THE PRODUCTION OF MUTATIONS IN DROSOPHILA WITH NEUTRON RADIATION

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EVER since the discovery of neutrons in 1932 by Dr. J. CHADWICK of the Cavendish Laboratory, Cambridge University, difficulties have stood in the way of the use of neutrons in the biological field. In the first place, it has been difficult to produce them in sufficient intensities. Secondly, nearly all of the neutron sources that might have been used not only emit neutrons of very heterogeneous energies, but also emit intense gamma radiation whose wave lengths vary through wide ranges. Moreover, the gamma radiation from most neutron sources is completely inseparable from the neutron radiation, so that the biological effects due to the neutrons and to the gamma rays cannot usually be separated by the use of gamma-ray control experiments. Finally, measurements of the intensities of neutron beams, or even determinations of their orders of magnitude, are attended by serious experimental difficulties. Through physical research, these drawbacks have been partly overcome, although there still remains much to be accomplished. Meanwhile, great care must be taken both in the performance and in the interpretation of biological experiments with neutrons.

Very few experiments with neutrons in the field of biology have been reported. LAWRENCE and LAWRENCE (1936) have found that fast neutrons are approximately ten times as biologically effective as X-rays in altering the blood picture of rats. ZIRKLE and AEBERSOLD (1936) performed experiments to determine the comparative effects of X-rays and fast neutrons on wheat seedlings. Fast neutrons were found to be about twenty times more effective than the equivalent dosage of hard X-rays in retarding growth in wheat seedlings.

The only genetic experiment with neutrons that has come to the attention of the writers is that of WHITING (1936) on the production of dominant lethals in the parasitic wasp, Habrobracon. The data presented however, as the author states, are meager.

The possible efficacy of neutrons in causing mutations was suggested by one of us in a recent paper (LOCHER 1936). The genetical aspect of the present problem was carried out under the guidance and suggestions of Dr. EDGAR ALTENBURG to whom we wish to express our sincere thanks and appreciation. We wish to thank the AMERICAN ONCOLOGIC HOSPITAL at Philadelphia for the use of the 4-gram radium bomb, through the courtesy

of Dr. G. M. DORRANCE, the director, and Dr. F. E. ULRICH, of the RICE INSTITUTE, for working out the probable errors in the results.

A preliminary account of this experiment has been reported in Nature (NAGAI and LOCHER 1937).

PRODUCTION OF NEUTRONS

Neutrons, like alpha particles, exist only in atomic nuclei and must be ejected from them if they are to be used. Neutrons for this experiment were obtained with the "radium bomb" of the AMERICAN ONCOLOGIC HOSPITAL, (NEWCOMET and HUGHES 1935), by allowing part of the gamma radiation from 4 grams of radium to pass through 485 grams of beryllium metal in a cylinder 8.0 cm in diameter and 9.0 cm in height. The radium holder, containing eighty 50-mg needles, was put in contact with one end of the beryllium cylinder, inside the massive lead bomb.

Gamma-rays whose energies exceed 1.6 MEV (millions of electron volts) can disintegrate beryllium nuclei, with the emission of neutrons, by the "photo-nuclear effect"; the neutrons thus obtained are sometimes called "photo-neutrons." In the present case, two gamma-ray lines of the RaC—RaC′ spectrum (1.76 MEV and 2.20 MEV, respectively) are capable of effecting this disintegration. The former line predominates in intensity. The kinetic energies of the neutrons are, therefore, $1.76-1.6=0.16$ MEV (or 160 kilovolts), and $2.20-1.6=0.60$ MEV (or 600 kilovolts), respectively. The total emission (in all directions) from the present source is calculated to be, roughly, 200,000 neutrons per second.

It is to be expected that the chief biological effects of fast neutrons will arise from the ionization along paths of recoil protons (hydrogen nuclei) that they eject by elastic collisions. The maximum ranges, in air, of protons ejected by unscattered neutrons from the present source are, according to the Cornell University energy-range curves of 1937, 0.22 cm and 1.1 cm, respectively. In water, the corresponding ranges are, approximately, 2.2 microns and 11 microns, respectively. Most of the recoil protons will, of course, have much smaller ranges, since their paths are oblique to the directions of incidence of the impinging neutrons.

Lead is rather transparent to fast neutrons, since the fraction of the kinetic energy lost by a neutron at each scattering decreases with the atomic number of the scattering element (1.9 percent, maximum, for lead, as compared with 100 percent maximum, for hydrogen). On the other hand, the scattering cross-section (or the probability of scattering) of the fast neutrons increases slowly with atomic number. The action of the thick lead shield provided by the wall of the radium bomb is, then, to introduce considerable diffusion of the (fast) neutron beam, without great absorption of energy from it. The lead intervening between the source and the point

of observation produces scattering-out of the neutrons, but all other lead in the vicinity produces scattering-in.

Figure 1 shows the arrangement of the neutron source and flies during irradiation. Two groups of flies, (F) and (S), were simultaneously irradiated with fast and with slow neutrons, respectively. The flies were placed as close to the beryllium block as was compatible with the retention of an adequate thickness of lead for the absorption of the gamma radiation.

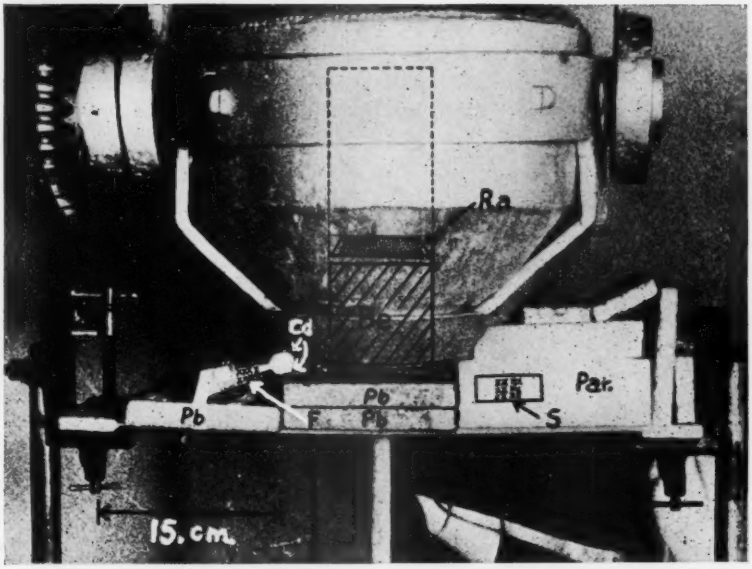

FIGURE 1.—Arrangement of apparatus during irradiation of flies with photo-neutrons. The radium (Ra) and beryllium (Be) are in a central hole of the lead bomb. The flies (F), irradiated with fast neutrons, are protected from very slow neutrons by a cadmium screen (Cd). A paraffin box (Par.) slows down the neutrons, by scattering, for irradiation of another group of flies (S) with slow neutrons. Lead blocks (Pb) and the lead and steel of the radium bomb absorb most of the γ-rays from the radium.

The distance of the perpendicular from the "line of centers" of the radium bomb to the mid-position of the flies in the vials was 12.6 cm. The distance from the center of the beryllium source to the mid-position of the flies was 11 cm. A cadmium filter 0.023 cm in thickness was used to filter out the very slow neutrons that may reach the flies as a result of scattering in beryllium and paraffin. The scattering in the beryllium, however, has been shown to be negligible. The flies thus protected by the cadmium filter received the "fast" neutron treatment.

The "slow" neutron flies were placed at the same distance from the

beryllium source in a paraffin box which weighed 4.5 pounds. The paraffin serves to slow down the neutrons by multiple scattering with hydrogen; its use for this purpose is general practice in neutron experiments. The paraffin facing the beryllium source was 1.2 cm in thickness, while that on all other sides of the box presented a thickness of at least 2.4 cm to neutrons incident from the beryllium source or scattered into the box by the lead of the bomb. The side of the box farthest from the bomb was about 5.0 cm thick and served as a backward-scatterer for the neutrons. This arrangement was chosen for its relatively high yield of slow neutrons, on the basis of a previous series of experiments with the production of artificial radioactivity in a special Geiger-Müller counter with a silver cathode. The distance of the "slow" neutron-treated flies from the beryllium source was approximately the same as that given for the "fast" neutron flies.

The terms "fast neutrons" and "slow neutrons" are, of course, only relative, in the same sense that "hard X-rays" and "soft X-rays" are relative terms. The fast neutrons used here are liberated with two distinct kinetic energies which are probably not greatly altered before the particles reach the flies. The slow neutrons, however, undoubtedly have a continuous range of energies that extend from those of the unscattered neutrons to zero, mainly as the result of scattering in the paraffin. The total number of fast neutrons in the "slow" group might be roughly 25 percent as great as in the fast neutron group.

Since some gamma radiation from the radium accompanies the neutrons, and since exact physical measurements of its biological effectiveness cannot be made, gamma-ray controls are necessary, especially in view of the fact that gamma-rays cause mutations (HANSON and HAYS 1928; HANSON and WINKLEMAN 1929). A paraffin "dummy" of the same dimensions as those of the beryllium source was used in place of the latter. It should give about the same amount of scattering of the gamma radiation as does the beryllium, without emission of an appreciable number of neutrons. Two sets of gamma-ray controls were run; one set with the flies in paraffin and the other in air. The latter should have had a little more gamma radiation than the one kept in paraffin; however, the difference in effect between the two should be almost negligible.

Ionization measurements of gamma-ray intensity at the position of the flies, both in neutron-irradiated and gamma-ray control groups, were made. A small ionization chamber (12 cc volume, with argon at 10 atmospheres) was connected through a semi-flexible cable to a vacuum-tube electrometer; the latter was used as a null-method instrument. The ionization current measured at the position of the flies was 50 micro-micro-amperes as compared with a current of 1.91 micro-micro-amperes due to ionization

by 1.00 mg of radium in a single needle 10 cm from the center of the chamber. The radiation on flies would therefore, be 50/1.91 or 26.2 mg Ra equivalent at 10 cm, or at 1 cm, 0.26_2 mg Ra equivalent. Taking TAYLOR'S (1936) value of 8.6 roentgens per mg Ra at 1 cm from a point source of Ra, the radiation on the flies per hour would be $8.6 \times 0.26_2 = 2.25$ roentgens. Thus, the flies exposed to 36 hours of irradiation (the usual treatment in these experiments) received 2.25×36 or 81 roentgens.

Certain classes of slow neutrons may be measured, but it is not as yet possible to measure the intensity of either the slow or the fast neutrons falling on the flies in these experiments. We hope to measure the neutron flux from the source we used by a new method now under development.

PRODUCTION OF MUTATIONS

To determine the effect of neutrons on the mutation rate, adult eosin males of *Drosophila melanogaster* were treated and lethals in the sex chromosome looked for. The lethals were detected by MULLER'S ClB method. One treated male was used per bottle in the P_1, and the F_1 Bar females were reared in vials. The lethals were located with regard to *w*^{e} *v f*. For convenience a lethal in the region between *w*^{e} and *v* is indicated as I, and between *v* and *f* as II in the tables. The symbols I^s and II^s indicate semi-lethals in these regions. Inv. indicates an inversion.

The eosin stock flies used in all of the experiments in any particular series were of the same kind. They were taken from several different bottles, mixed well, and then divided into three separate vials for each of the four experiments in each series. The three groups of flies were, namely, for (1) the neutron-treated, (2) the corresponding gamma-ray controls, and (3) the non-irradiated controls which did not receive any kind of radiation. They all received identical handling except during treatment period. The neutron-treated flies and the corresponding gamma-ray control flies were not exposed to their respective radiations at the same time. They were usually exposed alternately, for equal intervals of 3 to 8 hours.

Three series of experiments were carried out with the Ra-Be source of neutrons previously described. The first series, or the preliminary series, consisted of experiments A, B, C, D; the second, experiments E, F, G, H; and the third series, experiments I, J, K, L. The data for each series will be considered separately, then the summarized data will be given.

The results of the first series of experiments are shown in table 1. Experiments A and C met with accident and no data were obtained from them. The gamma-ray controls of experiment D died (in transport from Swarthmore to Houston). The dosage of neutrons in these experiments was low (3 to 9 hours), since it was not yet known what the maximum dosage was that the flies could tolerate (or that could be administered under the

TABLE 1

EXP. NO.	TREATMENT	NO. HRS.	NO. MALES MATED	NO. MALES STERILE	NO. MALES TESTED	NO. F_1 MATED	STERILITY NO.	STERILITY %	NO. F_1 TESTED	LETHALS NO.	LETHALS %	CLASSIFICATION AND LOCATION FROM DIFF. MALE	CLASSIFICATION AND LOCATION FROM SAME MALE	MUTATION RATE
B	Fast Neutrons	6	23	2	21	993	42	4.22	951	6	.63	4(II, I, II, I)	2(II^s, II)	1 in 158.5
XB	Gamma rays	6	17	—	17	741	39	5.26	702	4	.56	($2I^s$, I)	2(II, II)	1 in 175.5
CB	No Radiation	—	18	1	17	729	48	6.58	681	1	.15	1(II)	—	1 in 681.0
D	Fast Neutrons	9	5	1	4	226	10	4.42	216	1	.46	1(II^s)	—	1 in 216.0
XD	Gamma rays	9	—	—	—	—	—	—	—	—	—	—	—	—
CD	No Radiation	—	12	—	12	593	34	5.73	559	3	.53	3(II^s, I, II)	—	1 in 186.3

TABLE 2

EXP. NO.	TREATMENT	NO. HRS.	NO. MALES MATED	NO. MALES DEAD	NO. MALES STERILE	NO. MALES TESTED	NO. F_1 MATED	STERILITY NO.	STERILITY %	NO. F_1 TESTED	LETHALS NO.	LETHALS %	CLASSIFICATION AND LOCATION FROM DIFF. MALE	CLASSIFICATION AND LOCATION FROM SAME MALE	MUTATION RATE
E	Slow Neutrons	16	12	—	1	11	691	68	9.84	623	1	.16	1(I)	—	1 in 623.0
XE	Gamma rays	16	18	—	1	17	863	115	13.32	748	3	.40	3(II, I, I)	—	1 in 249.3
CE	No Radiation	—	19	1	2	16	729	49	6.72	680	3	.44	3(II, I, I)	—	1 in 226.0
G	Fast Neutrons	16	21	—	—	21	1969	87	4.42	1882	18	.96	5(II^s, II, II, I, I)	3(II^s, II, II) 2(II^s, II^s 2(I, II^s) 2(II, I) 2(I, I) 2(II^s, II)	1 in 104.5
XG	Gamma rays	16	22	—	1	21	1658	104	6.27	1554	6	.39	6(I, II, II^s, II^s, II, I)	—	1 in 259.0
CG	No Radiation	—	9	—	—	9	485	37	7.63	448	—	.00	—	—	0 in 448.0
H	Fast Neutrons	40	11	—	1	10	794	69	8.69	725	6	.83	4(II, II, ?, I)	2(I, II)	1 in 120.8
XH	Gamma rays	40	—	—	—	—	—	—	—	—	—	—	—	—	—
CH	No Radiation	—	8	—	1	7	264	15	5.68	249	—	—	—	—	0 in 249.0
CF	No Radiation	—	6	—	—	6	349	32	9.17	317	—	—	—	—	0 in 317.0

TABLE 3

EXP. NO.	TREATMENT	NO. HRS.	NO. MALES MATED	NO. MALES DEAD	NO. MALES STERILE	NO. MALES LOST	NO. MALES TESTED	NO. F_1 MATED	STERILITY NO.	STERILITY %	NO. F_1 TESTED	LETHALS NO.	LETHALS %	CLASSIFICATION AND LOCATION FROM DIFF. MALES	CLASSIFICATION AND LOCATION FROM SAME MALE	MUTATION RATE
I	Slow Neutrons	36	18	—	1	—	17	632	64	10.1	568	4	.70	4(I^s, II^s, I^s, II)	—	1 in 142.0
XI	Gamma rays	36	15	—	5	5	5	98	21	21.4	77	—	—	—	—	0 in 77.0
CI	No Radiation	—	13	—	1	2	10	573	40	6.98	533	4	.75	4(I^s, II, I, I^s)	—	1 in 133.2
J	Slow Neutrons	36	22	2	6	—	14	368	55	14.9	313	—	—	—	—	0 in 313.0
XJ	Gamma rays	36	19	1	6	1	11	406	107	26.3	299	—	—	—	—	0 in 299.0
CJ	No Radiation	—	22	2	3	1	16	1155	135	11.6	1020	—	—	—	—	0 in 1020.0
K	Fast Neutrons	36	22	—	4	—	18	856	122	14.2	734	8	1.09	3(II, I, II)	3(I, II, II) 2(II, I)	1 in 91.7
XK	Gamma rays	36	21	—	4	1	16	1093	135	12.2	958	3	.31	3(II, ?, ?)	—	1 in 319.3
CK	No Radiation	—	13	—	1	—	12	839	129	15.3	710	1	.14	1(II^s)	—	1 in 710.0
L	Fast Neutrons	36	22	—	2	—	20	1126	155	13.7	971	12	1.23	6(I, II, II, I, ?, I)	2(II, II) 2(Inv, I) 2(II, II)	1 in 80.9
XL	Gamma rays	36	24	1	4	—	19	1311	183	13.9	1128	7	.62	7(I II^s, II, I, II, I, I^s	—	1 in 161.1
CL	No Radiation	—	26	1	4	—	21	930	115	12.3	815	4	.49	4(I, v, II^s, II)	—	1 in 203.7

conditions of the experiment). The data are not significant. The data from this preliminary series of experiments are not included with the rest, in which larger dosages were employed, as it was not the purpose of the present experiments to determine how the effect, if any, varied with the dosage, but to determine whether there was an effect in the first instance, for which purpose the results got with the maximum tolerable dosage would be preferable.

TABLE 4

EXP. NO.	TREATMENT	NO. HRS.	NO. MALES TESTED	NO. F_1 TESTED	LETHALS NO.	LETHALS %	MUTATION RATE
G	Fast Neutrons	16	21	1882	18	.96	
H	Fast Neutrons	40	10	725	6	.83	
K	Fast Neutrons	36	18	734	8	1.09	
L	Fast Neutrons	36	20	971	12	1.23	
Total			**69**	**4312**	**44**	**1.02**	**1 in 98**
XE	Gamma-ray Con.	16	17	748	3	.40	
XG	Gamma-ray Con.	16	21	1554	6	.39	
XI	Gamma-ray Con.	36	5	77	—	—	
XJ	Gamma-ray Con.	36	11	299	—	—	
XK	Gamma-ray Con.	36	16	958	3	.31	
XL	Gamma-ray Con.	36	19	1128	7	.62	
Total			**89**	**4764**	**19**	**.398**	**1 in 250**
E	Slow Neutrons	16	11	623	1	.16	
I	Slow Neutrons	36	17	568	4	.70	
J	Slow Neutrons	36	14	313	—	—	
Total			**42**	**1504**	**5**	**.332**	**1 in 301**
CE	No Radiation	—	16	680	3	.44	
CG	No Radiation	—	9	448	—	—	
CH	No Radiation	—	7	249	—	—	
CF	No Radiation	—	6	317	—	—	
CI	No Radiation	—	10	533	4	.75	
CJ	No Radiation	—	16	1020	—	—	
CK	No Radiation	—	12	710	1	.14	
CL	No Radiation	—	21	815	4	.49	
Total			**97**	**4772**	**12**	**.25**	**1 in 398**

The results of the second two series of experiments, in which larger dosages were used (16 to 40 hour treatment) are shown in tables 2 and 3, respectively, and the combined data in table 4 in more condensed form. A summary of all the results is as follows:

SERIES	NO. OF F_1 BRED	NUMBER OF LETHALS			MUTATION RATE
		TOTAL	1 PER MALE	2 OR MORE PER MALE	
1. Fast neutrons	4312	44	18	26	0.01021±0.00103
2. Slow neutrons	1504	5	5	0	0.00332±0.00100
3. Gamma-rays	4764	19	19	0	0.00399±0.00062
4. No radiation	4772	12	12	0	0.00252±0.00049

The combined results got with the fast neutrons are definitely significant in comparison with the gamma-ray controls. The difference between the frequency rates in 1 and 3 is six times the probable error in 1 and ten times the probable error in 3. The slow neutrons, on the other hand, give no significant result in comparison with the gamma-ray controls, the difference in the two rates being only 0.62 times the probable error in 2 and 1.8 times the probable error in 3. The gamma-rays are having a slight effect. The difference between the frequency rates in 3 and 4 is 2.3 times the probable error in 3 and 3.0 times the probable error in 4.

The number of lethals per male is noteworthy. The total number of males that yielded lethals in the fast neutron-treated series was 25, of which 11 yielded two or more lethals apiece (giving the total of 26 shown in the above summary). This contrasts sharply with the results of the gamma-ray controls and the remaining series, in that there was no tendency for any such grouping of the lethals in them.

Only one inversion was obtained in the present series of experiments. The inversion, located in region I (between w^e and v) was found among the flies treated for 36 hours with fast neutrons (table 3).

DISCUSSION

It is not surprising that slow neutrons produced no effect, since these would not as a rule produce dense tracks of ions in matter containing a large proportion of hydrogen, as is true of living tissue. Any hydrogen atoms that absorbed a neutron would give off very short-wave radiation, similar to gamma-rays, and this would as a rule pass out of the tissue without causing any change, if the tissue were very small in volume (although the gamma-rays might sometimes happen to produce ions in the tissue and thus cause mutations). The carbon and nitrogen in living tissues would not be disintegrated by neutrons. Any boron or lithium that was present in the tissues would be broken down with the liberation of alpha particles and protons, and these would produce mutations by means of the dense tracks of ions that they produce. However, there is not much boron or lithium in living tissues ordinarily. The physiological effects produced

in rats (LAWRENCE and LAWRENCE 1936), it will be recalled as well as the retardation of growth in wheat seedlings (ZIRKLE and AEBERSOLD 1936), were due to fast neutrons.

The grouping of the lethals in the fast neutron-treated series calls for special comment. Since adults were treated, the grouping could not be due to "reduplication" of lethals produced in embryonic or germ tract cells. Apparently, the only possible interpretation of these results is that if a neutron produces any "hit" at all, it tends to strike in more than one place. The vast majority of the neutrons would, of course, not be absorbed or deflected and would pass right through the narrow limits of the sperm cells without producing any effects on them. Those that are deflected within the mass of sperm cells would produce a track of ions among the sperm cells (in the path of the recoil protons), and it is to be expected that this track might produce more than one mutation, since the distance between ions is very short (in comparison with the distance between ions produced by gamma-rays).

A large part of the effect of fast neutrons must depend upon the presence of hydrogen in the tissues and be due to the short-range, densely ionizing paths of the recoil protons. Hydrogen, however, is not the only element that can act as a scatterer. Recoil atoms of other elements found in the protoplasm may play a part; especially is this likely if the neutrons are of very high velocity. If there is any direct relation between the biological effectiveness of ionizing particles and the specific ionization of the particles, it is probable that recoil atoms of elements other than hydrogen may affect that process. So we cannot assume that the biological effects of fast neutrons are confined to the effects of recoil-protons. Ionization resulting from collisions of neutrons with other elements may appreciably affect the part of cells which may be especially sensitive to ionization.

SUMMARY

Fast neutrons are capable of producing mutations, as indicated by the fact that in the treated series, 44 lethals appeared in 4312 F_1 females, derived from 69 eosin males, a mutation rate of 1 in 98, as compared with 19 lethals in 4764 F_1 females, derived from 89 eosin males, a mutation rate of 1 in 250, in the gamma-ray controls. The lethal mutation rate of fast neutron-treated flies is therefore 2.6 times that of the corresponding gamma-ray controls. The difference between the frequency rates in the fast neutron series and gamma-ray controls is six times the probable error in the fast neutron series, and ten times the probable error in the gamma-ray controls.

Slow neutrons did not, under the conditions of the experiment, affect the mutation rate. Only 5 lethals appeared in 1504 F_1 females (obtained

from 42 eosin males), a rate of 1 in 300.8. The difference between the frequency rates in the slow neutron series and the gamma-ray controls was only 0.62 times the probable error in the slow neutron series, and 1.8 times the probable error in the gamma-ray controls.

There was a tendency for a treated male to produce two or more mutations, if he produced any mutations at all. Since adults were treated, this grouping of the mutations was not due to "reduplication" of mutations produced in the early germ tract, but to separate mutations. This would indicate that there was a tendency for several mutations to occur in each track of ions caused by a neutron (in cases of "hits").

LITERATURE CITED

HANSON, F. B., and HAYS, F. M., 1928 The effects of radium in producing lethal mutations in *Drosophila melanogaster*. Science **68**: 115–116.

HANSON, F. B., and WINKLEMAN, E., 1929 Visible mutations following radium irradiations in *Drosophila melanogaster*. J. Hered. **20**: 277–286.

LAWRENCE, JOHN H., and LAWRENCE, ERNEST O., 1936 The biological action of neutron rays. Proc. Nat. Acad. Sc. **22**: 124–133.

LOCHER, GORDON L., 1936 Biological effects and therapeutic possibilities of neutrons. Amer. J. Roentgen. **36**: 1–13.

NAGAI, MARY, and LOCHER, GORDON L., 1937 Production of mutations by neutrons. Nature **140**: 111–112.

NEWCOMET, W. S., and HUGHES, B. A., 1935 Flexible radium bomb with adequate protection. Amer. J. Roentgen. **33**: 694–697.

TAYLOR, LAURISTON S., 1936 Measurements of X-rays and Radium. chapter II, p. 86 *Biological Effects of Radiation*, Vol. I. New York and London, McGraw-Hill Book Company, Inc.

WHITING, P. W., 1936 Dominant lethal genetic effects caused by neutrons. Science **84**: 68.

ZIRKLE, RAYMOND E., and AEBERSOLD, PAUL C., 1936 Relative effectiveness of X-rays and fast neutrons in retarding growth. Proc. Nat. Acad. Sci. **22**: 134–138.

THE GENETICS OF SELF-INCOMPATIBILITY IN OENOTHERA ORGANENSIS[1]

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OENOTHERA *organensis* is endemic to the Organ Mountains of southern New Mexico where the entire population apparently consists of considerably less than one thousand individuals.[2] The morphology of the seeds indicates a relationship to members of the subgenus Oenothera (sometimes called Onagra), but the growth habit and ovule placentation resemble types belonging to the subgenus Raimannia (the Euoenothera of Engler and Prantl). *Oenothera organensis* has proved completely cross-sterile with five species of Raimannia. Hybrids with Oenothera species are obtained infrequently, and are almost completely sterile, resembling Oenothera-Raimannia hybrids. *Oenothera organensis* differs from both these subgenera in being completely self-sterile.

The inheritance of self-incompatibilty in *Oe. organensis* proved to be of the Nicotiana type, governed by a series of multiple allelomorphs. Interest attaches not so much to the mode of inheritance as to the methods by which genetic analyses can be followed in this material.

MATERIAL

Seeds of *Oenothera organensis* were collected at Modoc Waterfall, at the mouth of Filmore Canyon on the western slope of the Organ Mountains by Dr. E. W. ERLANSON and Dr. A. E. ARCHER in November, 1928. Plants from these seeds were grown in Pasadena in 1929, 1930 and 1931, and were continued from a single open-pollinated capsule from one of these 1931 plants. The culture grown in 1934 from these 1931 seeds was the first to be tested for self- and cross-incompatibility. Ten plants tested proved to be self-sterile. Nine of these plants were pollinated by a tenth, eight being cross-fertile and one cross-sterile. The first experiments to be reported were made on the progenies of these cross-fertile combinations.

In 1935, Professor P. A. MUNZ collected seeds from plants growing in the type locality, Dripping Springs, in the next canyon south of Filmore Canyon. Professor R. E. CLELAND grew plants from these seeds in Balti-

[1] **Oenothera organensis** Munz n. nom. *Oe. macrosiphon* Woot. & Standl., Contr. U. S. Nat. Herb. 16. 155. 1913, not *Oe. macrosiphon* Lehm. in Hamb. Gartenz. XIV: 439. 1858.

[2] In September, 1937 there were 70 plants in one canyon at Dripping Springs, 42 plants in another canyon at Dripping Springs, less than 20 plants at Modoc Waterfall (June 1937), and about 20 plants in one fork of McAllister Canyon on the north slope of the mountains. Unless there are some unexpected stands of this species in other parts of the mountains, the total population must be less than 500.

more the following year, found that they were self-sterile and made intercrosses between seven of them. Seeds of these crosses were sent me and the progenies were tested at Pasadena in the summer of 1937.

CROSS-INCOMPATIBILITY IN ONE FAMILY

A culture of 21 plants was grown in the summer of 1936 from seeds of a cross between two members of the 1934 culture of the Modoc strain. Fifteen of these (including all then in flower) were intercrossed in nearly all possible combinations. The first results were extremely complicated: reciprocal crosses reacted alike in some instances and not in others. Following repeated pollinations, seeds were finally obtained in both or neither directions in most reciprocal crosses, and the family could be classified into four intra-sterile, inter-fertile groups. The data obtained are summarized in table 1.

TABLE 1

Results of cross-pollinations within one family. A set of seeds is indicated by the + sign, the failure of the capsule to set by the − sign.

	♀ \ ♂	TYPE A				TYPE B			TYPE C		TYPE D
		6	8	9	10	1	4	7	18	3	12
TYPE A	2	−−	−−	−	−	++	+	+++	−+	+	
	5	−−	−	−	−	+	+	+	+	+	++
	6	−−	−−	−	−−	+	+	++	++	+	++
	8	−−	−−−	−	−	+	+	++	+	+	++
	9	−	−−	−−	−	+	+	++	+	+	++
	10	−−	−−	−	−	−	+	+	+	+	++
	20	−	−−	−	−	+	+	+	−−	++	−
TYPE B	1	−+				−	−	−	+	+	−
	4	++	+	+	++	−		−	+	+	++
	7	−−+	++	+	+	−	−	−−−	−+	+	+
TYPE C	18	−						−	−−		
	3	++	+	+	++	+	+	+	−	−−−	++
	14	−	+			+	+			−	
	15	−−	+	++	++	−+	−		−	−	+
TYPE D	12	−+	++	+	++	+	+	++	+	+	−−

INCOMPATIBILITY TESTS WITHIN THE STYLAR TISSUE

Flowers which had been pollinated 24 hours earlier were removed from the plant, killed, the styles dissected and stained by the method of BUCHHOLZ (1931) for Datura. Following compatible cross-pollination, the

pollen tubes had just reached the ovaries (150 to 180 millimeters below the stigma), whereas following self-pollination the pollen tubes remained in the stigma and were mostly less than one millimeter in length.

On flowers removed from the plant and kept in a moist chamber at 25°C, pollen tubes from compatible pollinations grew at nearly a constant rate. Pollen tubes from self-pollinations apparently stopped growing after the first hour or two (fig. 1).

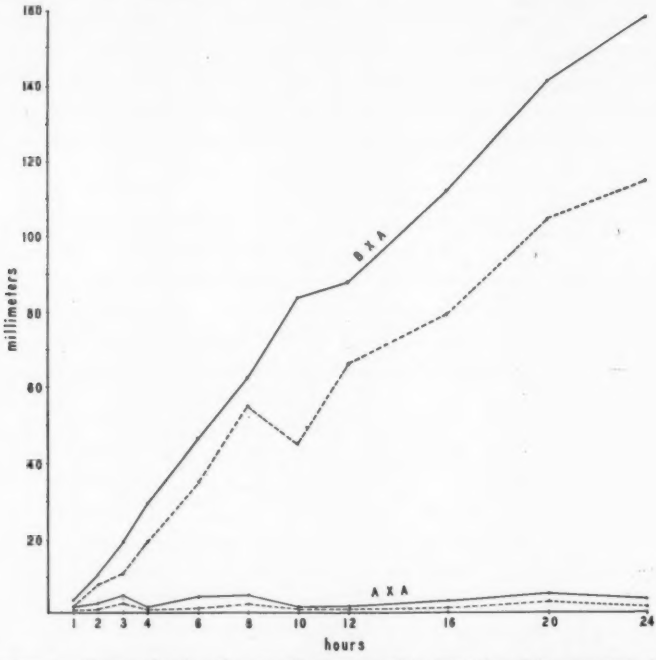


FIGURE 1.—Curves showing the growth of compatible (B×A) and incompatible (A×A) pollen tubes in the styles of flowers removed from the plants. The solid lines show the lengths of the longest pollen tubes, the dotted lines show the mean lengths of all pollen tubes in the styles.

The distinction between compatible and incompatible combinations is clearly evident after four hours, at which time the longest pollen tubes are still within the relatively thick portion of the style (approximately 1 millimeter in diameter) where dissections are readily made. All subsequent tests for cross-incompatibility have been made by this method. In figure 2 the results of tests made in the summer of 1936 are summarized. In these tests the time varied from four to five hours and the temperature from 20° to 27°C. The tests are generally very clear, with the exception of occasional flowers in which incompatible pollen tubes grew longer than is customary,

and in such flowers very few pollen tubes reacted in this way. Consequently, tests made by this method proved much more reliable than those in which the failure of seeds to set was used as a criterion of incompatibility.

The culture which had previously been tested by the set of capsules (table 1) proved to have 8 plants of type A, 5 of type B, 4 of type C and 3 of type D, and there was one plant that failed to flower. Other cultures grown in that year were not tested as completely, but only one culture contained plants belonging to a different incompatibility group. This culture had 8 plants of type C and 3 of type E, with 11 that failed to flower.

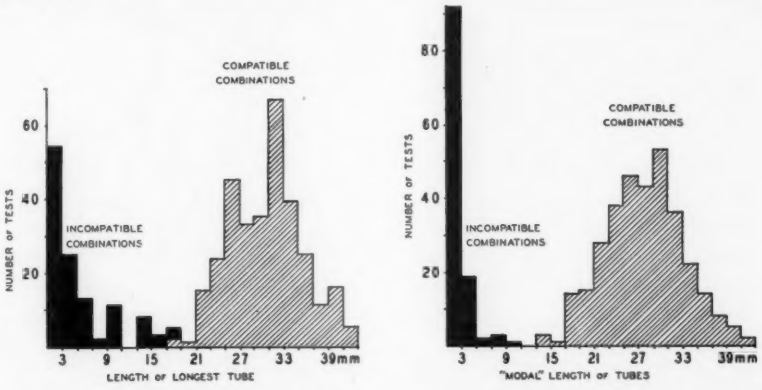


FIGURE 2.—Distribution curves of pollen tubes of compatible and incompatible combinations 4 to 5 hours after pollination. The modal length was determined by inspection, not from measurements of all tubes.

PROGENY TESTS

Intercrosses between the different incompatibility groups were grown in the greenhouse in the winter of 1936–37 where their flowering was induced by artificial illumination (long day treatment). The frequencies of the various incompatibility types appearing in these cultures are summarized in table 2, to which have been added data from similar crosses grown in the summer of 1937.

The data indicate that a series of multiple allelomorphs are responsible for self-incompatibility in *Oenothera organensis*, as in Nicotiana (EAST and MANGELSDORF 1925) and many other plants. The action of these allelomorphs is such that pollen carrying any particular allelomorph fails to produce normally developing pollen tubes in styles heterozygous (or homozygous) for that allelomorph. In those crosses in which the parents had completely dissimilar allelomorphs, four sterility types are expected

TABLE 2

Frequencies of different incompatibility types in the progenies of intercrosses between types A, B, C, D and E.

CROSS	GENOTYPES OF PARENTS	TYPE A S_1S_2	TYPE B S_3S_4	TYPE C S_1S_3	TYPE D S_2S_4	TYPE E S_2S_3	TYPE F S_1S_4
A×B	$S_1S_2 \times S_3S_4$	.....	.....	5 4 6 6	4 0 1 2	3 0 3 9	9 1 2 1
B×A	$S_3S_4 \times S_1S_2$	.....	.....	1	1	1	1
A×C	$S_1S_2 \times S_1S_3$	.....	.....	2 4 10	.....	3 2 16	.....
C×A	$S_1S_3 \times S_1S_2$	1 2 7 9 5	.....	.....	.....	1 1 3 9 4	.....
A×D	$S_1S_2 \times S_3S_4$	.....	.....	.....	3 0	.....	2 1
D×A	$S_3S_4 \times S_1S_2$	3	.....	.....	.....	.....	0
B×C	$S_3S_4 \times S_1S_3$	.....	.....	5 2	.....	.....	1 6
C×B	$S_1S_3 \times S_3S_4$	.....	1	.....	.....	.....	2
B×D	$S_3S_4 \times S_2S_4$	.....	.....	.....	1	1	.....
D×B	$S_2S_4 \times S_3S_4$	.....	0	.....	.....	1	.....
C×D	$S_1S_3 \times S_2S_4$	1 0	2 0	.....	.....	2 2	0 0
D×C	$S_2S_4 \times S_1S_3$	2	0	.....	.....	1	3
E×A	$S_2S_3 \times S_1S_2$	12	.....	4	.....	.....	.....
E×B	$S_2S_3 \times S_3S_4$	.....	7	.....	4	.....	.....
E×C	$S_2S_3 \times S_1S_3$	10	.....	12	.....	.....	.....
E×D	$S_2S_3 \times S_2S_4$	.....	13	.....	7	.....	.....

among the offspring, none of which represents either parental type. In those crosses in which the parents had one allelomorph in common, only two sterility types are expected among the offspring, and one of these should be identical to that of the male parent.

The allelomorphs responsible for the present types may be determined by the following method:

The cross A×B yields four types, C, D, E and F, hence A and B can have no allelomorph in common. A may be taken as S_1S_2, B as S_3S_4.

The cross A×C produces only two types, C and E, hence A and C have one allelomorph in common and this is taken as S_1.

The cross B×C also produces two types, C and F, and the allelomorph which B and C must have in common is taken as S_3. Then C has the constitution S_1S_3.

In the cross A (S_1S_2)×C (S_1S_3), only S_3 pollen functions, producing S_1S_3 which is type C and S_2S_3 which must then be type E.

In the cross B (S_3S_4)×C (S_1S_3), only S_1 pollen functions, producing S_1S_3 which is type C and S_1S_4 which must then be type F.

Since the crosses A×D and B×D each produce but two types including that of the pollen parent, D must have one allelomorph in common with type A and one with type B, and since C and D can have no allelomorph in common (the four types A, B, E and F are produced in intercrosses between them). D must be S_2S_4.

The remaining crosses listed in table 2 serve as a check on this interpretation. The o's included in this table indicate classes that should have occurred in the particular families but which were not observed.

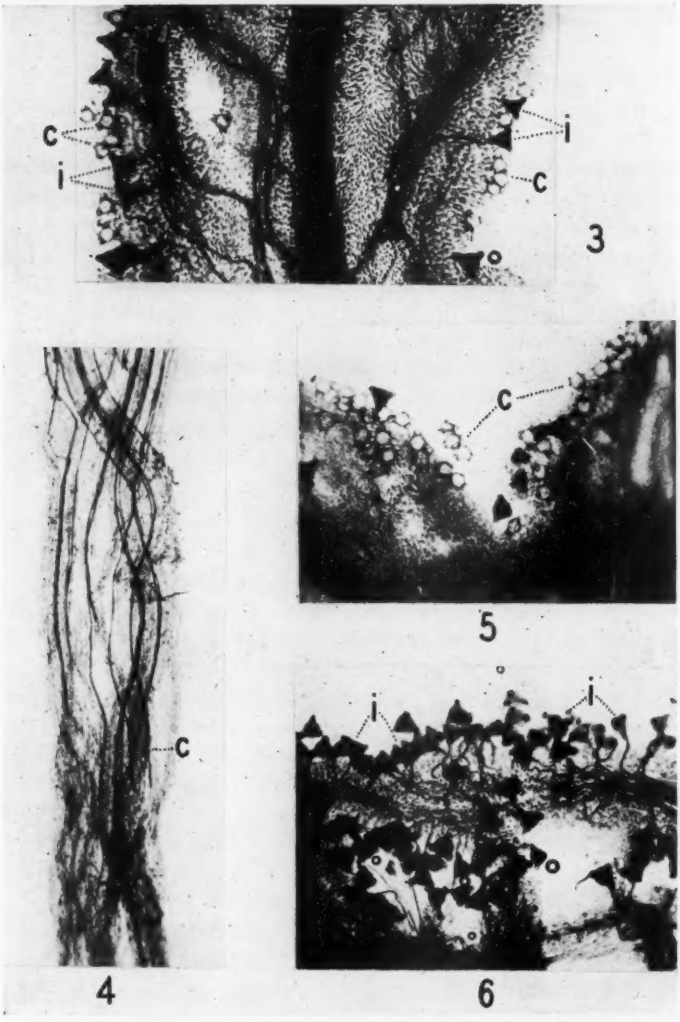
TESTS FOR ADDITIONAL ALLELOMORPHS IN THE GAMETOPHYTE GENERATION

An examination of stigmas prepared four hours after pollination showed that it is possible to distinguish between the two sorts of pollen in crosses between types having an allelomorph in common, for example $S_1S_2 \times S_1S_3$. Figure 3 is a photomicrograph of a stigma following this type of pollination. The incompatible pollen (carrying the allelomorph also present in the stylar tissue) produces very short tubes and the pollen grains retain their contents. The compatible pollen produces very long tubes (fig. 4) and the pollen grains and the upper portions of the tubes are empty and fail to stain. Figure 5 is a photograph of a completely compatible combination in which all of the germinating pollen has produced long tubes, the pollen grains becoming empty. Figure 6 is a photograph of a completely incompatible combination, equivalent to self-pollination, in which all of the germinating pollen produces short tubes and in which there are no empty pollen grains.

By using the presence of two types of pollen, that with long tubes and that with short, to indicate the presence of an allelomorph common to the two parents, it is possible to determine the genetic constitution of any particular plant without progeny tests. This method was used in analyzing the crosses obtained from Professor CLELAND (the Dripping Springs Strain). The data from these tests are presented in figures 7 and 8. Figure 7 is a table showing the behavior of pollen in intercrosses between the various incompatibility types occurring. Figure 8 is a table showing the incompatibility groups expected and the observed frequencies of plants belonging to these groups in the different crosses.

The details of the analysis follow:

Type O has one allelomorph in common with types C (S_1S_3) and E (S_2S_3) none with type A (S_1S_2), hence O has allelomorph S_3. Type O has no



FIGURES 3 to 6.—Photomicrographs of portions of prepared stigmas or styles four hours after pollination (magnification 22×). Figure 3, a partially compatible combination ($S_7S_8 \times S_8S_7$); c, compatible (S_8) pollen; i, incompatible (S_7) pollen. Figure 4, portion of style 20 mm below the stigma from the same preparation as Figure 3 to show ends of compatible pollen-tubes. Figure 5, completely compatible combination ($S_8S_7 \times S_9S_9$); the few stained pollen-grains failed to germinate. Figure 6, incompatible combination ($S_8S_8 \times S_8S_8$) equivalent to self-pollination.

allelomorph in common with type F (S_1S_4), hence the second allelomorph carried by O is different from all previously determined and is taken as S_5. Type O is S_3S_5.

Type G has one allelomorph in common with type O (S_3S_5), none with

♀ \ ♂	A 1/2	C 1/3	E 2/3	F 1/4	D 2/4	B 3/4	O 3/5	P 3/6	G 5/6	Q 3/7	J 5/7	H 6/7	M 5/8	S 6/8	V 7/8	T 5/9	L 6/9	N 7/9	K 8/9	Z 6/10	Γ 7/10	Φ 7/11
C 1/3		-	½	½	+																	
E 2/3		½	-	+	½																	
F 1/4		½	+	-	½																	
D 2/4		+	½	½	-																	
B 3/4	+	½			½	-																
O 3/5	+	½	½	+	+	½	-	½	½	½	½	+	½	+	+		+	+	+		+	+
P 3/6		½					½	-	½		+	½										
G 5/6	+	+	+	+	+	+	½		-			½										
Q 3/7	+	½				½			+	-	½	½	+					½				
J 5/7	+	+	+	+	+	+	½		½	½	-	½	½	+	½	½	+	¼	+	+	½	½
H 6/7	+	+	+	+	+	+	+		½	½	½	-	+	½	½	+	½	½	+	½	½	½
M 5/8									½		½	+	-	½	½				½			+
S 6/8							+		½		+	½	½	-	½		½	+	½			
V 7/8							+		+	½	½	½	½	½	-		+	½	½	+	½	½
W 3/9										½		+					½					
T 5/9							½	+	½		½	+					½		½			
L 6/9	+					+			½	+	+	½	+		+		-	½	½		+	
N 7/9	+	+	+	+	+	+	+		+	½	½	½	+	+	½		½	-	½	+		½
K 8/9			+	+					+		+	+			½				-			+
X 3/10										½		+										
Z 6/10	+	+	+	+	+	+			½		+	½	+	½	+		½	+	+	-	½	+
Γ 7/10	+	+	+		+	+		+	+				+		½			½	+	½		½
Θ 3/11										½		+										
Φ 7/11	+	+			+	+			+		½	½			½			½				

FIGURE 7.—Behavior of pollen-tubes in crosses between the different incompatibility types. The + symbol indicates a completely compatible combination, − a completely incompatible combination and ½ a combination with one allelomorph common to both parents. The letters in the headings refer to the incompatibility types, the fractions under or beside them refer to the allelomorphs of S carried by each type, for example, $\frac{3}{5}$ represents S_3S_5, etc.

types A to F (S_1 to S_4), hence G has S_5 and no other previously determined allelomorph. Type G is S_5S_6.

Type H has one allelomorph in common with type G (S_5S_6), none with type O (S_3S_5), hence H carries S_6 and no other previously identified allelo-

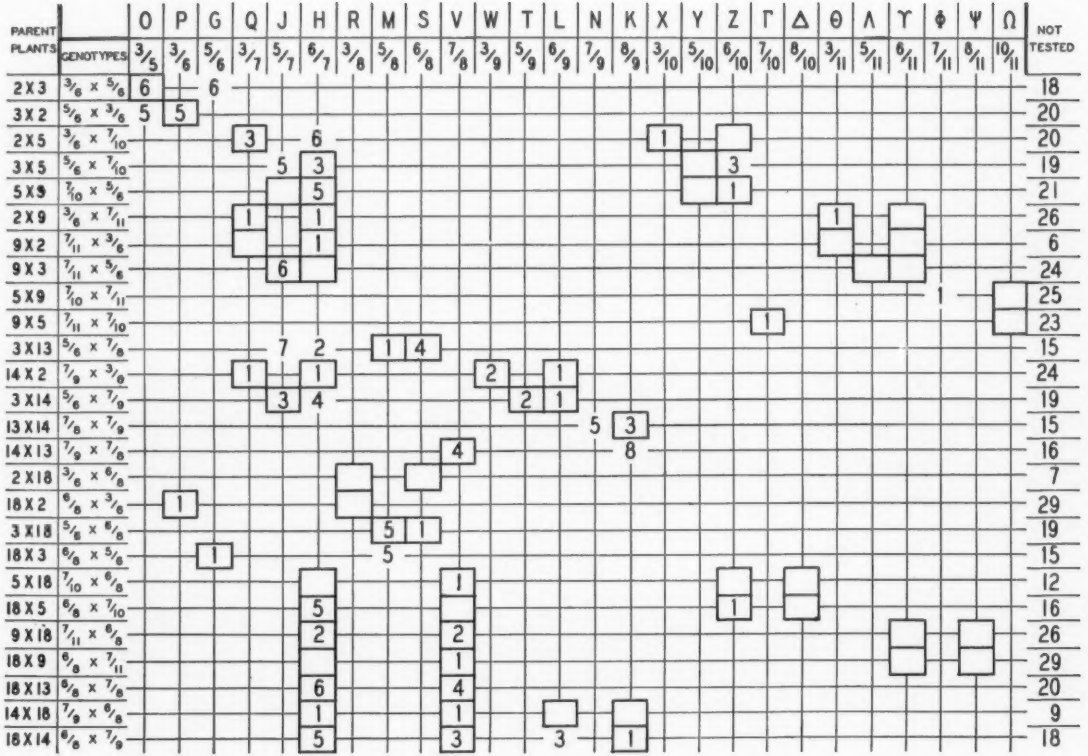


FIGURE 8.—Frequencies of different sterility types in different intercrosses. The heavy-lined squares in the table indicate predicted classes (see text).

morph, as shown by crosses with types A to F (S_1 to S_4). Type H is S_6S_7.

Type J has one allelomorph in common with each of types O (S_3S_5), G (S_5S_6) and H (S_6S_7). Type J does not have S_3 as it has no allelomorph in common with type C (S_1S_3). Hence J has S_5 in common with types O and G, S_7 with type H. Type J is S_5S_7.

Type K has no allelomorph in common with types E (S_2S_3), F (S_1S_4), G (S_5S_6) or J (S_5S_7). Type K is S_8S_9.

Type L has one allelomorph in common with type G (S_5S_6), none with type J (S_5S_7), hence L has S_6. Type L has one allelomorph in common with type K which can be either S_8 or S_9. Type L is S_6S_9.

Type M has one allelomorph in common with type J (S_5S_7), none with type H (S_6S_7), hence M has S_5. It has one allelomorph in common with type K (S_8S_9), none with type L (S_6S_9), hence M has S_8. Type M is S_5S_8.

Type N has one allelomorph in common with types J (S_5S_7) and L (S_6S_9) none with type G (S_5S_6), therefore N has S_7S_9.

Type Z has one allelomorph in common with type G (S_5S_6), none with type J (S_5S_7), hence type Z has S_6. Type Z has no allelomorph in common with types A to F (S_1 to S_4) nor with type K (S_8S_9). Type Z is S_6S_{10}.

Type Φ has one allelomorph in common with type J (S_5S_7), none with type G (S_5S_6), hence Φ has allelomorph S_7. Type Φ has no allelomorphs in common with types A (S_1S_2), B (S_3S_4), K (S_8S_9) or Z (S_6S_{10}). Type Φ is S_7S_{11}.

After these types had been identified, the genetic constitutions of the parents were deduced. It was then possible to predict the types that should occur in all intercrosses. The method of determining the constitutions of the parents is as follows:

Parent no. 3.

#2×#3 produced type G, #3 has S_5 or S_6 or both.
#3×#2 produced type O, #3 has S_3 or S_5, hence #3 is one of S_3S_6, S_5S_6, S_5S_z.
#3×#13 produced type H, #3 has S_6 or S_7, hence S_3S_6, S_5S_6 or S_5S_7.
#3×#13 produced type J, #3 has S_5 or S_7 not both, hence parent no. 3 is S_5S_6 or type G.

Parent no. 14.

#3×#14 produced type H (S_6S_7) and since S_6 came from #3, #14 has allelomorph S_7.
#3×#13 produced type H, hence #13 similarly has S_7.
#13×#14 produced type N (S_7S_9) and since #13 and #14 both have S_7, the pollen parent must be type N. Parent no. 14 is S_7S_9 or type N.

Parent no. 13.

#13 has been shown above to have S_7.

#14×#13 produced type K (S_8S_9) and since S_9 is from #14, parent #13 is S_7S_8, called type V.

Parent no. 5.

#3×#5 produced types J (S_5S_7) and Z (S_6S_{10}), and since allelomorphs S_5 and S_6 were from #3, parent no. 5 is S_7S_{10} called type Γ.

Parent no. 2.

#3×#2 produced type O (S_2S_5) and since S_5 is from #3, #2 must have S_3.

#2×#5 produced type H (S_6S_7) with S_7 from #5, hence parent no. 2 is S_2S_6 called type P.

Parent no. 9.

#9×#2 produced type H (S_6S_7) with S_6 from #2,

#5×#9 produced type Φ (S_7S_{11}) with S_7 from #9, parent no. 9 is S_7S_{11} or type Φ.

Parent no. 18.

#18×#3 produced type M (S_5S_8) with S_5 from #3,

#18×#14 produced type L (S_6S_9) with S_9 from #14, parent no. 18 is S_6S_8 called type S.

In figure 8, the heavy-lined squares indicate the additional classes expected in the various crosses. The numbers enclosed in these squares represent the observed frequencies of these classes. Unfortunately a fairly high proportion of the plants failed to flower during the summer so that the tests are necessarily incomplete. The data are sufficient, however, to serve as a reasonably good check on the above determinations.

CHROMOSOME CONFIGURATIONS

The culture of *Oenothera organensis* grown in 1931 directly from seeds collected at Modoc Waterfall contained plants with different chromosome configurations. The female parent of the 1934 culture had seven chromosome pairs. At least one other plant had a ring of six chromosomes and four pairs. Plants of the 1934 culture were not examined cytologically, but both configurations appeared in the 1936 culture derived from it. The configurations observed in the 1936 culture discussed earlier were:

Type A (S_1S_2)—{ 6 plants with 7 pairs; 1 plant with a ring of 4

Type C (S_1S_3)—3 plants with 7 pairs

Type D (S_2S_4)—3 plants with ring of 6

Type B (S_3S_4)—4 plants with ring of 6

The ring of 6 appeared whenever S_4 was associated with either S_2 or S_3. With the exception of the one plant with a ring of four chromosomes, 7 pairs resulted from associations of S_1 with S_2 and of S_1 with S_3. The further crosses made to test the association of the ring of 6 with S_4 are reported in table 3. While the tests are not extensive, all of the plants carrying S_4 (a total of 8) had rings of 6 chromosomes, whereas those with combinations of other allelomorphs (a total of 6) had 7 pairs of chromosomes.

TABLE 3

PARENTS	PROGENY
S_1S_2 (7 pairs) × S_2S_4 (ring 6)	1 S_2S_4 with ring of 6
S_1S_2 (7 pairs) × S_2S_4 (ring 6)	1 S_1S_3 with 7 pairs 3 S_1S_4 with ring of 6 1 S_2S_3 with 7 pairs 3 S_2S_4 with ring of 6
S_1S_3 (7 pairs) × S_2S_4 (ring 6)	1 S_1S_2 with 7 pairs
S_2S_4 (ring 6) × S_1S_3 (7 pairs)	1 S_1S_3 with 7 pairs
S_2S_4 (ring 6) × S_2S_4 (ring 6)	1 S_2S_4 with ring of 6
S_1S_2 (7 pairs) × S_1S_3 (7 pairs)	1 S_2S_3 with 7 pairs

The exceptional type A plant with a ring of 4 chromosomes (S_1S_2) was pollinated by a C-type plant (S_1S_3) with 7 pairs. Among the progeny, one S_1S_3 plant had 7 pairs, one S_2S_3 plant had a ring of 4, and three plants of the latter type had 7 pairs of chromosomes. These data, while extremely meagre, indicate that the S locus is independent of the ring of 4 chromosomes.

Both the ring of six chromosomes and the ring of four are invariably associated with the nucleolus. That the nucleolar chromosome does not contain the S locus is shown by the inheritance of the ring of four.

THE INHERITANCE OF MALE-STERILE

The 1936 culture previously discussed also segregated for male-sterile. In the male-sterile plants, meiosis proceeds normally, but the pollen grains produced are small and deficient in starch content and the anthers fail to dehisce.

Of the 20 plants in the 1936 culture, 5 were male-sterile. One of these belonged to type C (S_1S_3), four to type A (S_1S_3). Intercrosses between normal sibs showed that male-sterile is a simple recessive and that several of the normal plants were heterozygous for the gene (*ms*) involved. A B-type plant (S_3S_4) with a ring of 6 chromosomes and heterozygous for *ms* was backcrossed to an A-type (S_1S_2) male-sterile. Of the offspring carrying S_3, 14 were normal and 6 male-sterile; of the progeny carrying S_4, 2 were normal and 1 male-sterile. These results indicate that *ms* is independent of the S locus and of the ring of 6 chromosomes associated with S_4.

SUMMARY AND CONCLUSIONS

Self-incompatibility in *Oenothera organensis* is governed by a series of multiple allelomorphs in the same manner as in Nicotiana (EAST and MANGELSDORF, 1925). Pollen carrying any particular allelomorph fails to produce normally developing pollen-tubes in any style also carrying that allelomorph. The scheme was proved to hold for *Oe. organensis* first by progeny tests and secondly by tests of pollen-tube development in the tissues of the style and stigma.

The failure of seed production proved to be an unreliable criterion for determining cross-incompatibility, but direct tests of pollen tube development within the stigma proved entirely satisfactory. These results suggest that certain of the complications observed by SIRKS (1926) in Verbascum and by SEARS (1937) in Brassica may have been due to the inadequacy of the seed-set test for determining cross-compatibility.

The examination of pollen tube development directly within the stigma has a further advantage in that progeny tests are rendered unnecessary in establishing genetic constitutions.

In the studies to date, eleven allelomorphs have been established. Three of these were found only in material from Modoc Waterfall, seven only in material from Dripping Springs. Only one allelomorph occurred in both samples. An extensive test of the distribution of allelomorphs in the native populations is now under way.

Other characters studied in *Oenothera organensis* were chromosome configurations and male-sterility. Translocations involving three chromosomes are associated with allelomorph S_4 so that all combinations between S_4 and S_1, S_2 and S_3 have a ring of six chromosomes. All possible combinations between S_1, S_2 and S_3 have normally pairing chromosomes. A ring of four chromosomes appearing in a single plant proved to be independent of the S locus. Since both the ring of six and the ring of four are invariably associated with the nucleolus, S cannot be carried by the nucleolar chromosome. Male-sterile (*ms*) proved to be a simple recessive independent of the S locus and of the ring of six.

LITERATURE CITED

BUCHHOLZ, J. T., 1931 The dissection, staining and mounting of styles in the study of pollen tube distribution. Stain Tech. **6**: 13–24.

EAST, E. M., and MANGELSDORF, A. J., 1925 A new interpretation of the hereditary behavior of self-sterile plants. Proc. Nat. Acad. Sci. **11**: 166–171.

SEARS, E. R., 1937 Cytological phenomena connected with self-sterility in the flowering plants. Genetics **22**: 130–181.

SIRKS, M. J., 1926 Further data on the self- and cross-incompatibility of *Verbascum phœniceum*. Genetica **8**: 345–367.

SEX DETERMINATION IN A "BISEXUAL" STRAIN OF SCIARA COPROPHILA[1]

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INTRODUCTION

THE present investigation is concerned with a study of sex determination in a strain giving bisexual progenies which was derived from a species of fungus-gnat (*Sciara coprophila*) that typically gives only unisexual progenies. Previous studies have shown that in the species *coprophila* the sex of the individual fly is typically dependent upon the type of elimination of entire chromosomes from nuclei in the developing embryo, and that the type of elimination apparently depends upon the zygotic chromosome complex of the female parent. Hence, each female gives a unisexual progeny. In the bisexual strain of this species, a single female gives both sons and daughters, indicating that not only is sex dependent upon the zygotic chromosome constitution of the mother, but that some other factor enters in as well. Occasional progenies in the bisexual line were unisexual (male). This point will be discussed later. It seemed probable that a thorough investigation of this case might throw light on chromosome behavior and the sex problem in Sciara, and also on the general problems of sex determination.

METZ has used the terms monogenic and digenic to designate the two types of reproduction found in species of the genus Sciara. Females of the former type produce unisexual progenies, and those of the latter type, bisexual. The use of the terms is carried still further here to designate the type of individual female, that is, a producer of a unisexual progeny is called a monogenic female, and one producing a bisexual progeny, digenic.

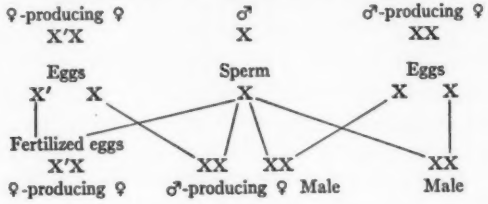
The earlier genetic and cytological studies on Sciara have been carried out in recent years by METZ and his associates. They have shown that genetically a female of a monogenic strain may have either of two chromosome constitutions. Her sex chromosome constitution may be X′X or XX. Flies with the former sex chromosome constitution are female-producing females, giving the two kinds of daughters in about equal numbers, while flies with the XX chromosome constitution are male-producing females. The evidence (METZ and MOSES 1928; METZ and SMITH 1931; METZ 1931a; METZ and ULLIAN 1929; METZ and SCHMUCK 1929a, 1929b) in-

[1] A dissertation submitted to the Board of University Studies of The Johns Hopkins University in conformity with the requirements for the degree of Doctor of Philosophy in the Department of Zoology, 1934.

dicates that all sperms are alike. Hence the sex of the progeny as a whole is dependent upon the zygotic chromosome constitution of the mother. In table 1 the mode of sex ratio determination is represented.

TABLE 1

Scheme of sex ratio determination in Sciara.



The sex of the individual fly depends finally upon the type of chromosome elimination which takes place during the early cleavage divisions of the developing embryo. The chromosome complex of the fertilized egg, whether it is to develop into male or female, is a small pair of V's, two pairs of rods, and a third rod of which there are three homologous members. Two of these three rods were brought into the fertilized egg by the sperm and are known to be sister halves of the maternally derived X in the male producing the sperm. In developing eggs from X′X females one of these sister halves of the X chromosome brought in by the sperm is eliminated (seventh or eighth cleavage division) from the soma cells and retained at least for a time by the nuclei making up the primordial germ cells. This leaves the soma with a pair of sex chromosomes. In developing eggs from XX mothers both sister halves of the sex chromosome from the sperm are regularly eliminated from the soma cells, leaving the soma XO in constitution. Eggs after such elimination develop into males.

In addition to the three pairs of autosomes and the X chromosomes there is a variable number, often a pair (but sometimes only one and sometimes three) of large V-shaped chromosomes which are carried by the germ cells. Since they are limited to the germ line, METZ has termed them the "limited" chromosomes. These are regularly eliminated from the soma. Apparently they play no significant part in sex determination and will, therefore, be disregarded.

Occasionally exceptional females appear in a male progeny and likewise exceptional males may appear in a female progeny. Under ordinary conditions such an exceptional female in a male progeny would have only the usual type of X and would certainly not carry the X′ factor. The bisexual line arose from one of these exceptional females.

METZ (laboratory notes) found that digenic females gave three classes of sons—suggesting the presence of three X chromosomes. Cytological

observations, however, revealed only two X chromosomes in ovarian cells. Since at that time nothing was known of chromosome elimination, the latter evidence was considered as conclusive indication that the digenic females were not XXX.

Dr. HELEN MONOSMITH PEARSON (unpublished data) made a study of this strain and suggested that each digenic female is a mosaic, from fertilization by two sperms, which could thus bring in two different X chromosomes. Later discovery of chromosome elimination put a new light on the subject and led to the present investigation.

MATERIALS AND METHODS

The material for the present investigation was a bisexual line obtained from Dr. C. W. METZ. Mutant stocks with which the flies were tested genetically were furnished from the same laboratory.

The flies were reared in glass vials ten cm in diameter. The cultures were kept at about 24°C. Evaporation from pans of water set in the chamber supplied sufficient moisture. The culture methods are those used by Dr. METZ and his colleagues in their work with Sciara (SMITH-STOCKING 1936).

Genetic methods

The results from Dr. METZ's laboratory which were at hand when this investigation was started in the spring of 1932 showed clearly that the sex chromosome was involved in the mechanism effecting the digenic reproduction. The results of the crosses of digenic females with truncate males had shown that the chromosome carrying the gene for truncate was not responsible for the bisexuality.

To test the other two linkage groups virgin females of the bisexual line were mated to males showing mutant characters, the genes for which are carried in one or the other of these two remaining chromosomes. Experiments were carried out with each of the two remaining linkage groups so that each of the two chromosomes was replaced in the bisexual strain by chromosomes from a monogenic line.

The sex chromosomes in the digenic females were replaced by X chromosomes carrying recessive mutant genes. If any one of these chromosomes was in any way responsible for the bisexuality of the line, it would not be possible except by intervention of crossing over to substitute completely chromosomes from the unisexual strains and to have the bisexuality continued. More details in reference to these tests will be brought out in the results.

Cytological methods

The cytological studies were made on whole eggs mounted in balsam. The technique used was developed in this laboratory in connection with the Sciara work (SCHMUCK and METZ 1931).

In order to get eggs in the desired stages of development, a special method of inducing flies to lay their eggs at a desired time was developed. Mated females were put into small glass vials which were one-third full of solidified agar-agar solution. After the agar-agar had shrunken away from the sides of the vial, the female, now two or three days old, was induced to crawl in between the agar-agar and the side of the glass vial, while the former was held back with a dissecting needle. The needle was then removed and the pressure of the agar-agar block on the thorax of the fly induced her to lay the eggs promptly. With this controlled method it was easy to get an abundance of eggs in any stage of development. (The eggs in Sciara are fertilized at the time of laying.) Eggs were fixed in Carnoy's 1:1:1 mixture. These were stained with fuchsin sulfurous acid (Feulgen's "Nuclealfärbung"). The staining technique as given by LEE (Ninth edition, page 437) was followed. The eggs were partially hydrolized in 1N HCl at 60°C for ten minutes. They were washed then in SO_2 water for three to five minutes, dehydrated, cleared with xylol, and mounted in balsam. It was necessary to leave the stained eggs in 80 percent alcohol overnight in order to remove the stain from the cytoplasm. After fixation and washing, the eggs were packed into Drosophila pupa skins to be carried through the various fluids. They were removed from the pupa skins in the balsam on the slide. The nuclei of the maturation divisions lie near the surface of the egg. By rolling the egg, effected by pushing the cover slip with a dissecting needle, the chromosome group could be viewed from various angles.

EXPERIMENTAL RESULTS AND DISCUSSION

As mentioned previously the autosome carrying the mutant gene truncate was shown by METZ and PEARSON not to play any part in the mechanism affecting bisexuality in the bisexual strain. Tests were made to ascertain whether or not either of the other two autosomes was responsible for the bisexuality of the line. The dominant gene Blister is carried in one of these autosomes and Curly, also dominant, in the other.

Virgin females from the bisexual line were out-bred to males from a monogenic line carrying Blister. All F_1 flies showed, of course, the mutant character. When such F_1 mutant females were out-bred to wild type males from a monogenic line, bisexual progenies were obtained showing wild type and Blister flies. In these mutant flies in the second generation both the chromosomes carrying Blister and its homologue were derived from monogenic lines. The digenic reproduction was retained and continued through at least two more generations at which time the lines were discontinued. Similar experiments with Curly showed conclusively that the chromosome carrying this gene could be entirely replaced with monogenic line chromosomes without interfering with the digenic reproduction.

From these results it may be concluded that only the sex chromosome need be taken into consideration.

Genetic evidence for trisomic germ line in digenic females

Evidence from genetic experiments shows that three X chromosomes are carried in the germ line of digenic females in the bisexual strain. Three recessive sex-linked mutant stocks were used in these experiments, swollen, round and miniature. Virgin females from the bisexual line were bred to males of one of the mutant stocks. F_1 virgin females were bred to males of the second mutant stock, and similarly F_2 virgin females were out-bred to males of the third mutant stock.

Wild type females in third generation progenies giving three classes of males (wild type, round, and swollen) were bred to swollen males. These swollen males came out of the experiments (male progenies), hence were not directly from a monogenic stock. Most of the progenies from these females again showed three classes of males, representing three possible combinations of three classes. They were wild type, round, miniature; wild type, swollen, miniature; and round, swollen, miniature. These results show conclusively that three X chromosomes were carried in the germ lines of the digenic mothers. The bisexual progenies showing these results are given in table 2.

Cytological evidence for the trisomic germ line in digenic females

The best picture of the chromosomes is found in the early part of the second maturation division. At this time the first polar body chromosomes are intact and the number of chromosomes in the germ line of the female can be ascertained.

Since it is seen from the genetic experiments that three sex chromosomes are being carried in the germ line of the digenic females, and since the evidence indicates that the sex chromosome in *coprophila* is one of the rods, an extra rod was expected in these eggs. Many eggs were examined and the extra rod was found. It will be remembered that the diploid number of chromosomes in the monogenic female is eight. In addition to the "limited" chromosomes there are one pair of small V's and three pairs of rods. Figure 1a shows a drawing of the chromosomes in the second oocyte, and figure

(a) (b)

FIGURE 1.—Chromosomes in second oocyte (a) and in first polar body (b) in one egg from a digenic female. Only the upper group of split chromosomes is represented. Feulgen preparation.

1b, those in the first polar body in a single egg from a digenic female. These are, of course, haploid groups. The chromosomes are already split

TABLE 2

Bisexual progenies showing three classes of males, representing three X chromosomes in the germ line of mothers. Progenies are grouped according to classes of males.

(a) Wild type (+), round (*r*), and miniature (*m*) males.

♀		♂			
+	*r*	+	*r*	*m*	*r m*
38		12	19	18	
59		6	28	23	
71		9	4	1	
7	1	2	5	7	
18	2	3	38	9	
44		19	38	18	4
47		11	32	31	
17		13	10	0	
301	3	75	174	107	4

(b) Wild type (+), swollen (*s*), and miniature (*m*) males.

♀			♂			
+	*s*	*m*	+	*s*	*m*	*m s*
62			10	9	4	
15		1	7	8	3	
41			31	33	20	
50			23	53	46	
50			47	50	27	
33	5		3	2	14	
5			3	8	7	2
10			10	17	13	
25			20	27	20	
54	1		34	34	29	1
64			8	15	15	3
9			16	29	12	
418	6	1	212	285	210	6

(c) Round, swollen, and miniature males.

♀				♂					
+	*r*	*s*	*m*	+	*r*	*s*	*m*	*r s*	*r m*
33	5			1	25	14	19	3	2
4				6	6	10	4		
63		1		1	24	18	23		
85		2		5	20	11	9		
55			2	4	26	4	33		3
47		21		10	8	13	2	4	
17				2	25	26	12	1	
53				3	26	5	16	3	
35				1	16	16	12		
43				4	12	13	3		
20				2	17	12	4		
7				3	11	1	0		
462	5	24	2	42	216	143	137	11	5

for the next division, but only the upper set in each case is shown here. It will be noted that the small autosomal V appears in both figures, 1a and 1b, but there are four rods in the oocyte figure and only three in the polar body. The extra rod seemed to pass at random into the polar body or into the second oocyte, as concluded after examining many eggs from a single female. In eggs from this digenic female only one limited chromosome was to be found. As is usual in *coprophila*, it was found to go either into the first polar body or into the second oocyte. In figure 1a it is with the five chromosomes in the second oocyte. In figure 2b it is seen with the five other chromosomes in the first polar body. Figures 2a and 2b were made from the second oocyte and the first polar body respectively, of another egg from the same female whose egg chromosomes are represented in figure 1. Here the two sex chromosomes (two of the rods) are passing out into the polar body, leaving one in the second oocyte, and hence one in the female pronucleus. Only the upper groups of the split chromosomes are represented.

(a) (b)

FIGURE 2.—Chromosomes in second oocyte (a) and in first polar body (b) in one egg from the same digenic female. Only the upper group of split chromosomes is represented. Feulgen preparation.

Selective segregation

The data obtained from experiments in which digenic females from successive generations were out-bred to mutant males from a monogenic stock seem to indicate that selective segregation obtains to an appreciable degree. Virgin digenic females were mated to males from a monogenic stock transmitting the mutant character round. Likewise virgin females from the first generation progenies were backcrossed to swollen males from a monogenic stock. Digenic females from these second generation progenies were out-bred to miniature males from monogenic stocks. The second generation progenies are recorded in table 3, third generation progenies in table 4 and fourth generation progenies are given in table 2.

From table 3 it is seen that only 19.46 percent of the second generation males showed the character round. Since round is sometimes carried concealed this percentage is undoubtedly a little low. But even after allowing for this inaccuracy the number of mutant males is still small for a random segregation of the three sex chromosomes carried in the germ line of the mothers of these flies. The character swollen, however, always shows in homozygous condition, and, as is evident in table 4, selective segregation obtains in maturation of eggs from digenic females carrying three distinc-

tive chromosomes, tagged with the wild type, round and swollen genes respectively. Here only 22.55 percent of the males in the third generation showed the swollen character. The class of miniature males in the fourth generation is not appreciably smaller than the other classes. However, outbreeding to miniature constituted the third successive generation in which

TABLE 3

Bisexual progenies from heterozygous round females mated to swollen males from a monogenic stock. Generation 2.

♀		♂	
+	r	+	r
8		33	9
82		0	0
74		23	4
55		21	6
23		25	3
13		49	3
37		86	12
20		16	9
61		50	5
44	1	80	21
30		62	23
11		7	1
27		24	2
32		17	7
49		51	14
13		5	4
17		25	7
32		27	5
36		25	9
14		6	2
53	2	65	33
54		11	9
59		56	11
58		17	6
11		2	2
23		62	5
13		33	0
16		17	0
65	1	31	7
22		16	12
49		21	4
1133	4	1080	261

digenic females from this line of experimentation were bred to monogenic stock males.

This type of behavior suggests that at maturation of the eggs synapsis between and separation of the two maternally derived chromosomes more often occurred, so that the paternally derived one, represented by the small class of males, more often accompanied one of the other two. In

19 to 22 percent of the cases, however, synapsis between and separation of the paternally and one of the maternally derived chromosomes took place, the other maternally derived X accompanying the latter, so that whatever force was at work here in bringing about this selection was not, by any means, absolute.

If selective segregation should account for a small class of males, the reciprocal class of females should also be small. So far as the evidence goes, the small classes of females support the hypothesis of selective segregation of the two maternally derived chromosomes.

TABLE 4

Bisexual progenies from females, recorded in table 3, mated to miniature males from a monogenic stock. Generation 3.

TYPE 1				TYPE 2					
♀	♂			♀		♂			
+	+	r	s	+	r	+	r	s	rs
28	31	0	7	40		39	19	15	1
19	13	0	11	14	1	4	4	5	
16	21	0	7	19		5	5	6	
20	23	0	0	20		9	5	2	
11	11	0	3	18		9	12	11	
10	9	0	2	13		9	3	1	
15	11	0	9	44		19	36	17	
10	11	0	2	35		32	15	13	1
38	28	0	2	60		10	10	11	2
11	30	0	7	29		48	45	0	
178	188	0	50	44		10	19	15	1
				50		0	40	14	1
				21		0	19	11	
				20		8	8	8	1
				17		17	16	4	
				17		9	10	4	
				13		8	6	4	
				20		10	5	6	
				19		5	5	6	
				11		5	6	5	
				18		14	17	7	
				28		19	11	6	
				40		27	24	13	
				610	1	316	340	184	7

The question is, what difference was there between the chromosomes of paternal derivation and the other two, maternally derived, or what similarity existed between the two of maternal derivation to cause this greater attraction of the two maternal chromosomes for each other. It seems that the most obvious difference is that for at least two generations the two maternally derived chromosomes had been together and subjected to the same cytoplasmic influences, while that from the father had been in this same environment for only a single generation. This might well

be sufficient to bring about a greater similarity and hence greater attraction between the two maternally derived X's. Since selective segregation against paternally derived chromosomes obtains completely in the first maturation division of male germ cells in Sciara (METZ 1927), it is not surprising that such selection as is described in the maturation of eggs in the trisomic germ line of digenic females should be found.

Why, in the fourth generation progenies (table 2), there seemed to be no particular selective segregation can be explained by the same sort of argument. After repeated out-breeding the environment in the cytoplasm of the germ line of the digenic female may not have been unique and sufficiently different from that found in the germ line of the monogenic males, so that no two chromosomes would come to be more similar and attractive to each other than any other two.

Bisexual factor

It has been mentioned that some females in the bisexual strain revert to male producers. This has been particularly true at certain times of the year, usually in midsummer and again in midwinter. It was observed that females in bisexual progenies which showed a mutant character were almost invariably monogenic. This fact suggested, after a trisomic condition in the germ line of digenic females had been demonstrated, that a gene carried in one of the original sex chromosomes of the bisexual strain was responsible for the retention of the trisomic condition.

To test this point digenic females were out-bred to swollen males from a monogenic stock. All flies of the first generation were wild type. Virgin females from these progenies were backcrossed to swollen males and gave in bisexual progenies wild type females and wild type and swollen males in the second generation. The males appeared in a ratio of two wild type to one swollen, with the swollen class deficient in number. More than two-thirds of these wild type females from the second generation, backcrossed again to swollen males from a monogenic stock, gave bisexual progenies consisting of wild type and swollen females and males in the approximate ratio of 2:1 and 1:2 respectively. Such a group of progenies is recorded in table 5.

Since the wild type females in such a progeny were digenic for the most part and the swollen females were monogenic, it was postulated that a bisexual factor in the wild type chromosome effected the retention of the trisomic condition of the germ line. To test this point further it was necessary to obtain by means of a crossover a bisexual line in which the bisexuality was linked with a definite mutant character. Such material was at hand in two all-swollen digenic lines which had been obtained in the course of the experiments and also in the bisexual progenies showing three classes of mutant males.

To ascertain in which of three sex chromosomes in the females represented in table 3c the bisexual factor might be carried, virgin females from these progenies were mated to wild type males from a monogenic stock and to the various mutant brothers. The mother of these progenies had mated to swollen males which came from male progenies in the various lines of the experiments. In the next generation 29 out of 51 progenies were bisexual. Here round, swollen and miniature females appeared as well as wild type females. These mutant and wild type females were again mated to wild type males from a monogenic stock and to the various mutant brothers. Only wild type and swollen females gave bisexual progenies in the next generation, and with one exception, all progenies from wild type females contained a class of swollen males. Many of these

TABLE 5

Bisexual progenies from heterozygous swollen females from second generation progenies, backcrossed to swollen males from a monogenic stock. Generation 3.

♀		♂	
+	*s*	+	*s*
19	7	10	16
59	29	14	32
36	6	24	26
40	13	31	45
15	2	10	12
28	13	10	15
32	3	25	32
28	21	14	20
64	23	4	14
18	4	8	20
339	121	150	232

bisexual progenies did not contain round and many did not contain a miniature class of males. No bisexual progeny was obtained from round or miniature females in any lines of the experiment. Since the bisexual factor must have been carried in one of the mutant chromosomes, and since swollen was the only mutant character common to all bisexual progenies (with the one exception), it may be concluded that the bisexual factor was now linked with that for swollen. The one exception to this was a progeny containing 44 wild type females, 16 wild type males, 12 round males and 18 miniature males. Sisters of the female giving this progeny gave bisexual progenies with a class of swollen males; therefore, her mother carried the swollen chromosome and hence the bisexual factor, if this chromosome was the one which carried the factor. This bisexual progeny may be accounted for in one of two ways: either, another crossover had occurred so that the bisexual factor was now carried in the wild type, round or miniature chromosome; or, without the presence of the

bisexual factor the tendency for the third sex chromosome to be eliminated from the germ line was not great enough to effect the elimination.

The second explanation brings up another point in connection with the effect of the bisexual factor. It will be recalled that many females in stock, particularly at certain times of the year, revert to male-producers. In the course of this investigation, females, known to carry the bisexual factor, were found to produce male progenies. We must postulate, then, another factor which affects the trisomic condition of the germ line, and which is opposed to the effect of the bisexual factor. This is a tendency for one of the three X's to be eliminated from the germ line. Whether this is a general tendency of the cytoplasm to reduce the triplo-X state or whether there are modifying factors in other chromosomes which oppose the bisexual factor in the X is not known. At any rate, such a variable tendency must be effective. If this tendency is high, the third X chromosome may be eliminated from the germ line of potentially digenic females in spite of the presence of the bisexual factor; or if it is low, the elimination may not occur when the bisexual factor is absent. The fact that many male progenies are produced in two seasons of the year indicates that the variation of the strength of this tendency may be correlated with the seasons, being highest in mid-summer and in mid-winter. The tendency must have been low in the following instance: a swollen female, from a progeny showing wild type and swollen females and wild type and swollen males in the ratios of 28:13 and 10:15 respectively, gave an all-swollen, bisexual progeny with 51 females and 51 males. All the females tested from this progeny gave typically male progenies. Here we may conclude that the tendency for elimination of the third X from the germ line of the mother of this all-swollen, bisexual progeny was not high enough to eliminate it even in the absence of the bisexual factor, but that such a tendency did effect the elimination in her daughters.

Swollen females from two other all-swollen, digenic lines, already mentioned, were mated to wild type males from a monogenic stock. They gave only swollen offspring (with occasional wild type daughters). The results in later generations seemed to indicate that the bisexual factor was now linked with swollen. The evidence at hand then seems to support strongly the hypothesis of the bisexual factor and the opposing tendency for the elimination of the third sex chromosome from the germ line of the digenic females.

It has long been known that males from the bisexual strain may be mated to monogenic females without showing any apparent tendency to transmit the bisexual factor. This behavior would be expected, however, since the factor is ineffective unless it appears in the female whose germ line already carries three X chromosomes. Such females are found normally in the bisexual strain and not in the monogenic lines.

Unisexual progenies

It has been stated previously that females in the digenic line may revert to male producers. Such females appear in too great numbers to be classed as ordinary exceptional females in male progenies. Male progenies from such females always show two classes of males, indicating that, some time after the fertilization of the XX egg which produced the mother of the male progeny, one of the three sex chromosomes must have been eliminated from the germ line. Almost without exception the chromosome eliminated was one of the maternally derived chromosomes, that is, the male-producing female always gave a class of males representing the sex chromosome from her father, the other class of males representing either one of the two maternally derived chromosomes.

Very rarely in the bisexual stock a female progeny appears. In the experimental lines of this investigation 19 definitely female progenies were obtained from females in bisexual progenies. This is 19 out of 984 progenies a percentage of 1.9, which is probably no greater than would be found in as many progenies in the bisexual stock. Previous tests of females from female progenies (notes from METZ's laboratory) showed that they were all unisexual producers. Most progenies obtained in these tests were male, but occasionally a female progeny was again obtained. The females in these second unisexual progenies were, however, male producers. From one of the female progenies obtained in these experiments (82 females: 14 males) 11 females were bred and gave three female and eight male progenies. Six females from one of these latter female progenies (45 females: 2 males) gave three typically bisexual and three male progenies. Daughters of these digenic females were bred and gave many bisexual progenies. The question arose as to why an occasional female in the digenic line would be a female producer. No adequate explanation has been found. The following possibility may account for such behavior: in the maturation of germ cells from the mother of a female progeny selective segregation may prevail so that always a single X chromosome is thrown off in the first polar body leaving two in the egg.

This possibility is somewhat borne out by the fact that a few male progenies were obtained that showed three classes of males. In these cases the reverse would have taken place leaving always the one X in the second oocyte. One male progeny consisted of 30 round, 8 miniature, 40 round-swollen, and 3 swollen flies. The mother must have been carrying round, miniature, and round-swollen. The three swollen males may have carried round concealed. Miniature came from the grandfather and the small size of this class was undoubtedly due to selective segregation, which usually prevails in digenic females out-bred to a monogenic stock. Other male progenies appeared to have come from females with germ lines carrying

three X's, but in them three classes of males could not be identified absolutely. Suggesting further that selective segregation may obtain, it is not at all uncommon to find bisexual progenies which show sex ratios approaching 1:0 or 0:1. An examination of sex ratios in progenies in table 2 shows that, when three classes of males are identifiable, the sex ratios may show an excessively large percentage of females or of males. It is not intended here to draw any conclusions concerning the reasons for obtaining female progenies, but rather to suggest the above possibility and to present the evidence at hand.

Chromosome elimination from somatic cells

The fact was mentioned earlier that in both females and males in the bisexual line the paternal sex chromosomes (two sister halves of the "precocious" chromosome) are eliminated from the somatic cells in early development. (This is the case also in the developing males in monogenic lines.) The fact that practically no swollen females were found in progenies from heterozygous swollen mothers, backcrossed to swollen, indicated this. Further evidence for this point was obtained from the progenies of digenic swollen females mated to wild type males. Table 6 shows a

TABLE 6

Progenies from four digenic swollen females mated to wild type males.

♀		♂	
+	*s*	+	*s*
0	6	0	9
3	9	0	38
1	23	0	27
1	20	0	27
5	58	0	115

series of such progenies. Here almost all the females were swollen and the two swollen chromosomes in the somatic cells must have been of maternal origin. Since it is known that the digenic female has three sex chromosomes in her germ line, this seems to be the most valid interpretation. Without this knowledge the plausible explanation would be on the basis of non-disjunction. Some indication of non-disjunction was found in a few cases in the course of the investigation, but these were rare.

From table 6 it is seen that five out of 63 females were wild type. One of two things may have happened to account for these wild type females. Either they came from one-X eggs and are, therefore, comparable to exceptional females in a male progeny, or they came from two-X eggs and one of the maternally derived X chromosomes was eliminated instead of of the usual paternal one.

Origin of the bisexual line

It has been shown by BRIDGES (1913) and others that non-disjunction of the X chromosome may occur in the maturation of the egg in Drosophila. As a result of this the female pronucleus contains two sex chromosomes instead of the usual one and when such an egg is fertilized by an X-bearing sperm, three X's are to be found in the fusion nucleus, a condition referred to as triplo-X. When such a fertilized egg develops, a rare occurrence in Drosophila, a superfemale is produced. The soma, as well as the germ line, is trisomic.

In the case of Sciara, where elimination of the paternal X's (sister derivatives of the "precocious" chromosome) from the somatic cells regularly takes place in eggs from XX females, which normally develop into males, and where one of these sister derivatives is regularly eliminated from the germ line, non-disjunction would bring about a three-X condition of the germ line, but the soma would contain two sex chromosomes of maternal origin, and would, therefore, be female. Although elimination of one of the paternally derived X's from the germ line has not yet been observed, one of these must be thrown out sometime during development. In order that, after non-disjunction, the two maternally derived X's and one of the paternally derived ones should be kept in the germ line, it seems, from the results of the present investigation, that the bisexual factor must be present in one of the sex chromosomes; otherwise one of the three X chromosomes is eliminated from the germ line, reducing the number to the usual two. Likewise it seems that if the bisexual line arose as a result of non-disjunction, the bisexual factor was carried in at least one of the X's of the original female.

Sex determination

In developing eggs from XX (not X′X) females in the monogenic lines of *Sciara coprophila* the two sister derivatives of the "precocious" chromosome are regularly eliminated, leaving the soma with one X, and, therefore, a male develops. It has been shown by this investigation that the usual type of elimination in eggs from XX females, that is, elimination of both halves of the "precocious" chromosome, prevails in developing embryos from digenic females of the bisexual line, whether these embryos are developing as females or as males. Since this type of elimination in the monogenic line brings about the development of the male, and since in the digenic line after the same type of elimination females as well as males regularly develop, it must be concluded that the sex of the individual here is dependent upon not only the type of elimination, but some other points as well.

In the course of this investigation it has been shown that the digenic

females carry three sex chromosomes in their germ lines, two of maternal origin and one of paternal origin. At maturation two types of ovotids are formed, one with two X chromosomes and one with one X. Females, then, develop after fertilization of ovotids which have two X chromosomes. It may, therefore, be concluded that in the digenic line the type of maturation, that is, whether one X or two X's appear in the ovotid, influences the sex of the resulting individual.

METZ (1934) has suggested that the two sister derivatives of the "precocious" chromosome brought into the egg by the sperm may be functionally different, that one may have been altered during the second spermatocyte division, when it was mechanically distorted, so that in the development of the fertilized egg it may function as a "Y." He suggested that in the germ line of developing male embryos the altered X (now a functional "Y") is retained, the unaltered one being eliminated, and that development of the testes depends on the chromosome constitution left, that is, X"Y". In the female embryos one of these sister derivatives of the "precocious" chromosome is eliminated from the female somatic cells, and METZ suggested that the one eliminated from the germ line may be identical with that eliminated from the soma, although the elimination from the germ line is much later in ontogeny. In the case of the female, it is suggested that the unaltered X or derivative of the "precocious" chromosome is retained both in the soma and in the germ line. If this be the true interpretation, the germ line and soma differentiate independently of each other as has been found to be the case in most insects.

The question arises as to what determines which of these two sister derivatives of the "precocious" chromosome is to be eliminated from the germ line; is it the same factor which controls the elimination from the soma, namely the zygotic chromosome constitution of the mother, or since elimination from the germ line is subsequent to that from the soma, is the chromosome constitution of the soma after elimination the controlling factor? If the type of elimination from both soma and germ line in males of the monogenic lines depends upon the same factor, namely the XX (not X'X) chromosome complex of the mother, then in the digenic line where the same type of elimination from somatic cells takes place in both females and males, the same derivative of the "precocious" chromosomes would be expected to be retained in the germ line of both sexes. If it is the altered half which is retained in the male, the one kept in the germ line of the female should also be the altered one. Yet this paternally derived X in the germ line of these digenic females acts as an ordinary X and not as a "Y," as indicated by its later behavior.

If, therefore, the two halves of the "precocious" chromosome are functionally different, it seems probable that the constitution of the soma after

elimination is the controlling factor determining which half is to be retained in the germ line. Since this differential idea was proposed to account for independent differentiation of the germ cells, it seems unnecessary if the type of elimination of chromosomes depends on the somatic chromosome constitution.

ACKNOWLEDGMENT

I am deeply indebted to Professor C. W. Metz for suggesting this problem, for his guidance and helpful advice throughout the investigation, for his generosity in furnishing flies from his laboratory, and for making accessible data on the "Bisexual" line of *Sciara coprophila* obtained by him and his colleagues.

SUMMARY

1. The germ line of the digenic females carries three sex chromosomes instead of the usual two.

2. At maturation two kinds of eggs are formed, one with two X chromosomes and the other with one. After fertilization, eggs of the former type develop into females and those of the latter type into males.

3. During the development of these three-X females the same type of elimination occurs as is found in the developing males from the XX male-producing females in the monogenic line; that is, the two sex chromosomes brought into the egg by the sperm are eliminated from the somatic cells and one is eliminated from the germ line of both males and females in the "bisexual" line.

4. There is a factor, the bisexual factor, in at least one of the sex chromosomes of the digenic female which is responsible for the retention of the trisomic (XXX) condition of the germ line.

5. There is a tendency for the third chromosome to be eliminated from the germ line of digenic females, which tendency opposes the effect of the bisexual factor.

6. At maturation of the eggs of the digenic female there is a tendency for synapsis to occur between the two maternally derived chromosomes.

LITERATURE CITED

BRIDGES, C. B., 1913 Non-disjunction of the sex chromosome of Drosophila. J. Exp. Zool. **15**: 587–606.

METZ, C. W., 1927 Chromosome behavior and genetic behavior in Sciara (Diptera). II. Genetic evidence of selective segregation in *S. coprophila*. III. Absence of parthenogenesis in *S. coprophila*. Z. i. A. V. **45**: 184–201.

1931a Chromosomal differences between germ cells and soma in Sciara. Biol. Zbl. **51**: 119–124.

1931b Unisexual progenies and sex determination in Sciara. Quart. Rev. Biol. **6**: 306–312.

1934 Evidence indicating that in Sciara the sperm regularly transmits two sister sex chromosomes. Proc. Nat. Acad. Sci. **20**: 31–36.

METZ, C. W., and MOSES, M. S., 1928 Observations on sex-ratio determination in Sciara (Diptera). Proc. Nat. Acad. Sci. **14**: 928–930.

METZ, C. W., and SCHMUCK, M. L., 1929a Further studies on the chromosome mechanism responsible for unisexual progenies in Sciara. Tests of exceptional males. Proc. Nat. Acad. Sci. **15**: 867–870.

1929b Unisexual progenies and the sex chromosome mechanism in Sciara. Proc. Nat. Acad. Sci. **15**: 863–866.

METZ, C. W., and SMITH, H. B., 1931 Further observations on the nature of the X-prime (X′) chromosome in Sciara. Proc. Nat. Acad. Sci. **17**: 195–198.

METZ, C. W., and ULLIAN, S. S., 1929 Genetic identification of the sex chromosome of Sciara (Diptera). Proc. Nat. Acad. Sci. **15**: 82–85.

SCHMUCK, M. L., and METZ, C. W., 1931 A method for the study of chromosomes in entire insect eggs. Science **74**: 600.

1932 The maturation divisions and fertilization in the eggs of *Sciara coprophila*. Lint. Proc. Nat. Acad. Sci. **18**: 349–352.

SMITH-STOCKING, HELEN, 1936 Genetic studies on selective segregation of chromosomes in *Sciara coprophila* Lintner. Genetics **21**: 421–443.

THE HOMOLOGY OF THE EYE COLOR GENES IN DROSOPHILA MELANOGASTER AND DROSOPHILA PSEUDOOBSCURA AS DETERMINED BY TRANSPLANTATION. II.

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INTRODUCTION

THERE are two methods by which chromosome and gene homologies can be studied: direct cytological examination, especially with the giant salivary gland chromosomes, and comparison of genetic chromosome maps. The first method is limited to those species which can be crossed and which produce viable offspring. Recent examples of the application of this method are the comparisons of salivary gland chromosomes of hybrids between *Drosophila pseudoobscura* and *D. miranda* (DOBZHANSKY and TAN 1936), and of *D. azteca* and *D. athabasca* (DOBZHANSKY and BAUER 1937). In species which cannot be crossed it is possible to apply only the genetical method. This involves establishing the homology of corresponding genes by the similarity of their phenotypical effects, and by comparison of their positions relative to other homologous neighboring genes. Attempts at such comparison were made early in Drosophila genetical work (MORGAN, BRIDGES and STURTEVANT 1925). More recently DONALD (1936) and STURTEVANT and TAN (1937) have used this method to make a detailed comparison of the linkage maps of *D. melanogaster* and *D. pseudoobscura*. The homology of genes in these species is based only upon the phenotypic effect. In a number of instances the comparisons are complicated because different genes produce similar effects.

We pointed out recently (1937) that the study of the reaction of eye discs in transplantation experiments can give useful information, especially for differentiating those genes which produce phenotypically similar effects. If in transplantation experiments the behavior of two genes is essentially the same, it may be concluded that they are homologous.

We have already established (1937) the homology of the genes "orange" *D. pseudoobscura* and "cinnabar" *D. melanogaster*, "vermilion" *D. pseudoobscura* and "vermilion" *D. melanogaster*, and "claret" *D. pseudoobscura* and "claret" *D. melanogaster*. It was pointed out that the two species differ quantitatively but not qualitatively in the reaction of implanted "wild type" anlagen. The behavior was always consistent with the supposition that *D. pseudoobscura* supplies less v^+ and cn^+ substances than does *D.*

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melanogaster. This is related to the fact that "wild type" *D. pseudoobscura* behaves in a manner identical to that of the recessive gene "clot" of *D. melanogaster*. We concluded that the gene "clot" or one similar to it is homozygous in *D. pseudoobscura* and is replaced in *D. melanogaster* by its "wild type" allele.

The present paper presents data obtained from intra- and inter-specific transplantation. It provides further evidence in support of the above conclusions and a general account of the behavior of all known eye color genes in *D. pseudoobscura*. On the basis of the new data we have established the homology of the known eye color genes in *D. pseudoobscura* and *D. melanogaster*.

METHODS AND MATERIAL

The methods used are described in our previous paper (1937). We have shown there that the difference in time of development of the two species is immaterial to the results obtained, as the host controls the period during which the implant undergoes pigmentation. The list of eye color mutants used in the present study is given here in alphabetical order with the standard mutant symbols:

D. pseudoobscura

ca—claret
cn—cinnabar
mg—magenta
or—orange
p^2—pink-2 (pink-allele)
rb—ruby
se—sepia
st—scarlet
v—vermilion
w—white
w^e—eosin (white allele)
+—"wild type" *D. pseudoobscura* (Texas, race A)

D. melanogaster

bw—brown
ca—claret
car—carnation
cd—cardinal
cl—clot
cm—carmine
cn—cinnabar
g—garnet
Hn^r—Henna^r (recessive allele of *Hn*)
p—pink
p^p—peach (pink allele)
rb—ruby
se—sepia
st—scarlet
v—vermilion
w—white
w^e—eosin (white allele)
$w^a cn$—apricot (white allele) cinnabar (double recessive)
$w^a v$—apricot vermilion (double recessive)
+—"wild type" *D. melanogaster* (Oregon-R-c)

Descriptions of these mutant genes are given by MORGAN, BRIDGES and STURTEVANT (1925) for *D. melanogaster* and by STURTEVANT and TAN (1937) for *D. pseudoobscura*. We shall refer to the mutant genes only by their symbols, but since the same symbol has often been used in both species indiscriminately, we shall distinguish the *D. melanogaster* and *D. pseudoobscura* symbols by the suffixes (m) and (p) respectively.

All of our *D. pseudoobscura* strains, with three exceptions, contained only a single mutant gene. The exceptions are: *pr*(p), carrying also beaded, yellow, short and Bare; *se*(p), carrying also beaded, miniature and snapt; and w^e(p) carrying also echinus and yellow. Since none of these affects the color of the eyes, their presence presumably did not affect the results. These stocks are therefore referred to by the symbols for the eye color only.

The stocks of *D. pseudoobscura* belong to race A, with the exception of "cinnabar," "orange" and "scarlet" which belong to race B.

THE BEHAVIOR OF THE EYE COLOR GENES AS DETERMINED BY TRANSPLANTATION

Table 1 presents diagrammatically the results obtained by the study of the eye transplants. The donor was always a female larva in its third instar (prepupa stage), the host a female or a male larva of the same age. Black discs in the table indicate a non-autonomous development of pigmentation. That means that the implant develops an eye color similar to that of the host, or in the cases of *v*(p), *v*(m), *or*(p), *cn*(m), *rb*(p), *g*(m) and *car*(m) develops the "wild type" eye color. Unshaded circles indicate autonomous development of pigmentation. Discs with a black sector ($\frac{1}{8}$-$\frac{7}{8}$) indicate, a partly non-autonomous development, where the implant is intermediate in color between the two controls; or in the seven mutants listed above, that the implant is intermediate between the "wild type" and the control implants in the donor larvae themselves.

Because of the complex interrelations between the different eye color genes considered in this paper, we have arranged the data in seven groups (a–g). The genes in each group have (a) identical relationship with each other; (b) the same type of relationship with the genes of any other group; (c) or apparently differ only quantitatively in either of the two properties. In table 1 these groups are separated by double lines.

The behavior of the eye color genes of D. pseudoobscura in intraspecific transplantation experiments

a) The + group. If + and w^e eye discs are implanted into different hosts, the developing eye is affected by *ca*, *mg*, *pr*, and p^2 in such a way that the final pigmentation is very like the host eye color (*ca* and *mg*), or

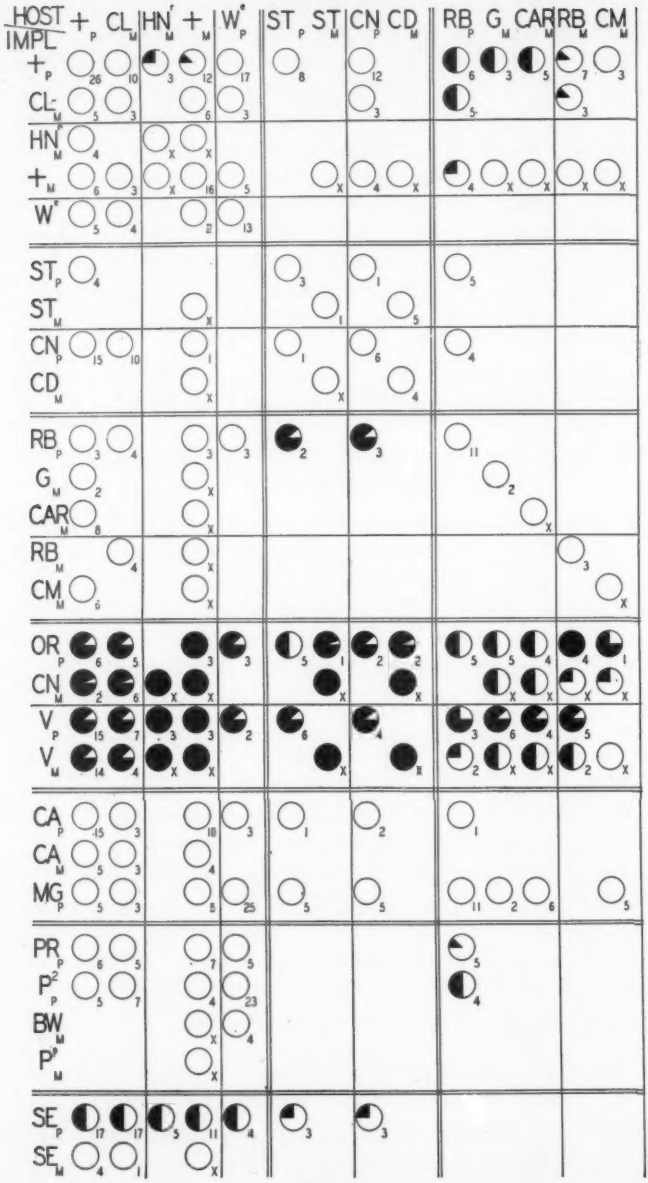

TABLE 1

The sign X means experiments done by BEADLE and EPHRUSSI. *For description see text.*

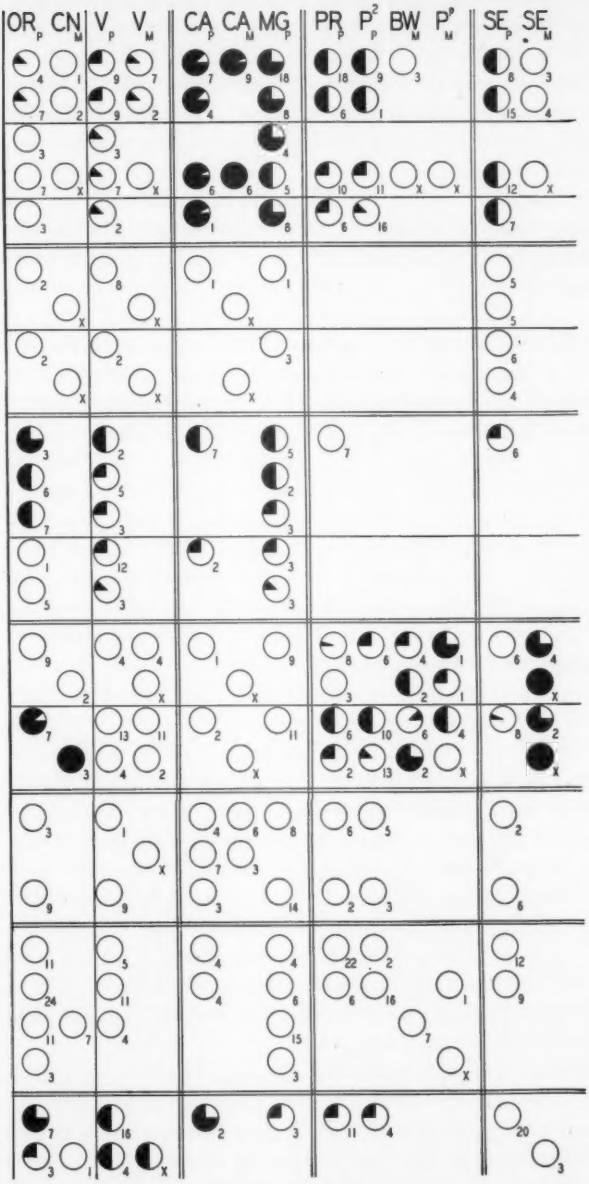

TABLE 1 (*continued*)

intermediate between the host and donor eye colors (*pr* and p^2). The light w^e implant does not change in *pr* amd p^2 as much as + does. In *or* and *v* hosts the + implant is changed to a slightly but distinctly brighter eye color than + controls. The change is more pronounced in *v* than in *or*. The w^e implant remains unchanged in *or*, but is visibly changed in *v*. In *se* hosts, the + and w^e implants develop a pinkish eye color, and become similar in appearance. + implanted in *rb* becomes intermediate between + and *rb*, and in *st* and *cn* it develops the original + eye color. It must be stated, however, that some of the eyes implanted into *cn* were brighter than the "normal" + implants. It is not clear from these experiments what effects age differences (younger and older + anlagen in *cn* hosts) might have.

The implantation of the eye discs of different mutants into + and w^e results in an autonomous development of the eye color in all cases except those involving *or*, *v* and *se*. The data given in table 1 for these three genes show that when implanted into + and w^e hosts, *v* and *or* optic discs give rise to a not quite completely "wild type" eye. Eye discs carrying the gene *se* develop in + and w^e hosts, a new dark brownish-red eye color. The new eye color resembles neither the + nor the w^e nor the *se* type, and apparently the amount of pigment in the new eye color type is not decreased.

+ in w^e, and the reciprocal combination, develop autonomously. Discussion of the interrelations of host and donor will be postponed until we have referred to the other *D. pseudoobscura* groups, and to the homologous groups in *D. melanogaster*.

b) *The st group*. The *st* and *cn* implants behave autonomously in all experiments carried out by us.

If the host is *st* or *cn*, then *rb*, *or*, *v* and *se* implants develop non-autonomously. The behavior of + in *cn* has been mentioned above. If *rb*, *or* and *v* discs are implanted into *st* and *cn* they are changed almost completely to "wild type" eyes. With the exception of *or* in *st*, the different combinations are indistinguishable, and similar to the implants of *or* and *v* in the + group. The eye discs carrying the gene *or* implanted into *st* become distinctly lighter than *or* in +. A change in the direction of the new eye color obtained from *se* implanted into + (see + group) occurs also in the experiment *se* implanted into *st* or *cn* hosts.

The development is autonomous in the experiments *st* implanted into *cn* and in the reciprocal combination.

c) *The rb group*. As an implant the *rb* eye shows an autonomous development in the + group and in *pr*. In the *st* group it changes almost completely to "wild type." The *rb* implant in *or* becomes very like "wild type." The *rb* optic discs give rise in *v*, *ca* and *mg* to an eye color which is intermediate between ruby and + eye colors. In *se* the implanted *rb* anlage is definitely pale, resembling neither *rb* nor +.

Implantation of + into a *rb* host resulted in a non-autonomous development, and an eye color very similar to that of *rb* implanted into *v*. In the experiment *or* implanted into *rb* the implant eye color is like that of + in *rb*, but more reddish, and *v* implanted into *rb* is very close to "wild type." In *rb* hosts the p^2 implant shows a "brownish-red" eye color similar to but not as dark as *se* implanted into+(see+ group). The eye color of *pr* implanted into *rb* is similar to, but lighter than, *se* implanted in *st* or *cn*.

d) *The v group*. We discuss *or* and *v* eye color genes together because in all cases in which the *or* eye discs give rise in the different *D. pseudoobscura* hosts to eye colors similar to "wild type," the *v* optic discs also develop non-autonomously to a + type. Nevertheless there is a distinct difference between the two genes, which cannot be explained by quantitative differences between them. Namely, *v* implanted into *or* gives rise to a "wild type" eye color; *or* implanted into *v* develops autonomously.

In +, w^e, *st* and *cn* hosts optic discs carrying the genes *or* and *v* develop to eyes which are not completely "wild type." The implant *v* in *rb* becomes more nearly "wild type" than does *or;* the same is true for *v* and *or* implanted into p^2, but here the change is not as great as in the preceding case. In *pr* the implanted *v* eye is indistinguishable from the implant of *v* in p^2. However, the *or* anlagen develop autonomously in *pr*, if the implanted eye is of normal size. If the implanted optic discs give rise to small eye pieces a change to + is clearly noticeable. That means that *pr* supplies sufficient cn^+ substance for the change to take place if the implanted eye is small. In *ca* and *mg* both *or* and *v* discs develop autonomously. In *se* only the *v* anlage shows a slight change to +.

If *or* and *v* are used as hosts, *st*, *cn*, *ca*, *mg*, *pr* and p^2 optic discs develop autonomously. + shows a slight change to a brighter eye color in *or*, and a more marked change in the same direction in *v*. The anlage carrying w^e changes only in *v*, and here to a lighter eye color. In *or* hosts the implant *rb* gives rise to a nearly "wild type" eye color; in *v* this change is not as strong and the eye is brighter. The implant *se* shows in *or* hosts an eye color which resembles very much *se* in +, but the implanted eye appears brighter. The color of the implant *se* in *v* is best described as intermediate between *se* and *v*. It has a slight orange tinge.

If *v* is implanted into *or* the anlage develops an eye identical to *v* in + host; *or* is autonomous in *v*.

e) *The ca group*. Our experiments have shown that *ca* and *mg* always develop autonomously when used as implants.

If *ca* and *mg* are used as hosts, only the implanted optic discs of the +, *rb* and *se* groups are non-autonomous in their development. The results of the experiments concerning +, w^e and *rb* are mentioned above. If *se* is implanted into *ca*, then the eyes show a reddish color closely resembling

ca itself; implanted into *mg*, *se* eyes show a more pinkish color than the *se* controls.

In reciprocal *ca-mg* implantations the optic discs develop autonomously.

f) *The pr group.* The behavior of *pr* and p^2 resembles very much that of *ca* and *mg*. However, the development of *pr* and p^2 implanted in *rb* hosts is not entirely autonomous (see above: the *rb* group).

If *pr* and p^2 are the hosts, + and w^e implants do not change as much as in the *ca* group. Eye discs carrying *se* implanted into *pr* or p^2 develop an eye color similar to that of *se* in *mg*. The behavior of optic discs carrying *or* or *v* when implanted in *pr* or p^2 is different from their behavior in *ca* or *mg* hosts. In the former cases they develop non-autonomously. The data are given above under d, the *v* group.

In the reciprocal experiments *pr* implanted into p^2 and p^2 implanted into *pr* the anlagen behave autonomously.

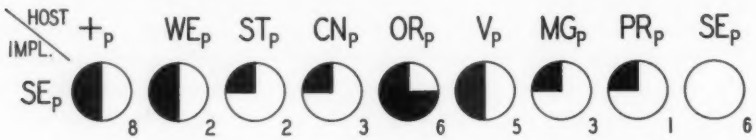

TABLE 2

Pupal dissections of se(p) optic discs implanted into different hosts (see text).

g) *The se group.* The behavior of the gene *se* is different in every respect from that of the other genes. If *se* is implanted into the + group the anlage gives rise to a new dark "brownish red" eye color. About the same eye color develops if *se* discs are implanted into the *st* group, but in this case the color is somewhat more *se*-like. In *or*, *se* changes to a color which is brighter but essentially of the same type as in +; in *v* it changes to an intermediate color (see above, *d*, the *v* group). In *ca* the eye color of the *se* implant proved to be more like that of the host than in *mg*, *pr* and p^2. Because the *se* eye darkens very rapidly with age, we have always examined freshly hatched flies, and in a number of cases dissected out pupae shortly before they hatched. The data obtained from experiments of the latter kind are listed in table 2. The results here agree with those presented in table 1.

The development by the + and w^e implants of a pinkish eye color is obvious in *se* hosts; *v* implanted into *se* hosts shows a slight change toward the + type. The other genes, with exception of *rb*, which has a pale eye color when implanted, behave autonomously in *se* hosts.

The observed behavior of all the genes within each of the seven groups listed above is *similar* in all experiments. In this sense, we may regard them as natural groups; only the *v* group is perhaps a composite. A com-

parison of the groups with each other is presented in table 3. The conclusions that may be drawn from the comparison are as follows.

a) The eye anlagen of the + group develop a color similar to that of the hosts when implanted into the *ca*, *pr* and *rb* groups; a new eye color in the *se* group, and a color distinctly brighter than normal in the *v* group. The development of the + group is autonomous only in the + group itself, and in the *st* group.

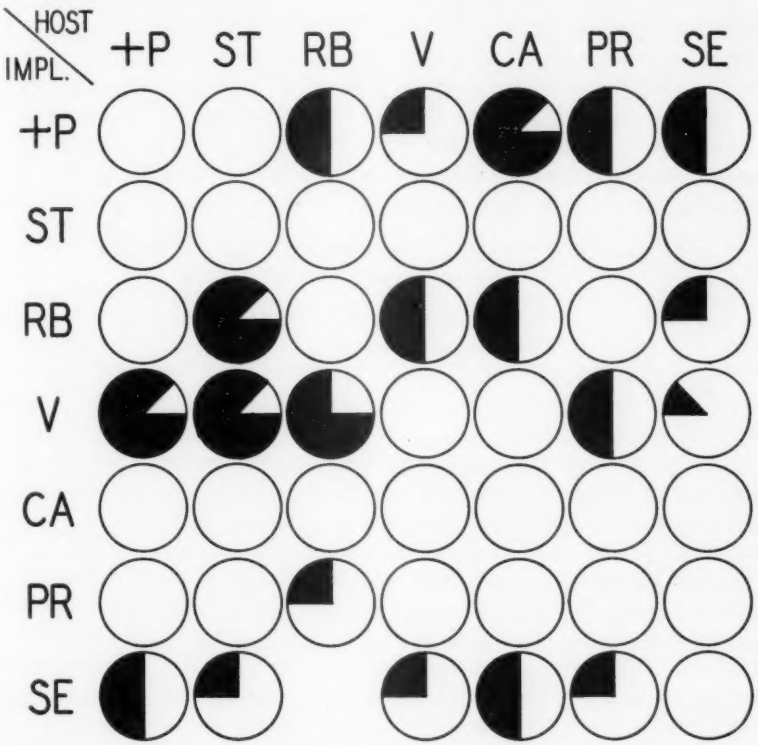

TABLE 3

Review of the behavior of the eye color gene groups within D. pseudoobscura (see text).

b) The *st* group always behaves autonomously.

c) The optic discs of the *rb* group implanted into the *st*, *v* and *ca* groups give rise to an eye color similar to the "wild type." The anlagen of the *rb* group develop a pale eye color when implanted into the *se* group. The eye discs carrying genes of the *rb* group develop autonomously only in hosts belonging to the *rb*, + and *pr* groups.

d) The *v* group shows a wild type eye color when implanted into the +, *st*, *rb*, *pr* and *se* groups; the development is autonomous only in the *ca* group.

e) The *ca* group always behaves autonomously.

f) The eye discs carrying genes of the *pr* group develop autonomously in all combinations studied, except when implanted into hosts belonging to the *rb* group. In the latter case, the implants develop an eye color resembling that of + implanted into *se*.

g) The *se* group changes the original color toward a dark "brownish-red" one in the + group, and to nearly the same color in the *st* group. When *se* is implanted into *v* the color of the implant becomes intermediate between *se* and *v*. The eye discs carrying genes of the *se* group, when implanted into *or* hosts, develop an eye color which is somewhat similar to that developed by the same implants into + hosts. In the *ca* and *pr* groups the color of the *se* implants is somewhat similar to that of the host eyes.

THE HOMOLOGOUS EYE COLOR GENES IN D. MELANOGASTER AND D. PSEUDOOBSCURA

a) The + group [+(*p*), *cl*(*m*), *Hn*^{r}(*m*), +(*m*), *w*^{e}(*p*), *w*^{e}(*m*)]. In our first paper (GOTTSCHEWSKI and TAN (1937), we pointed out that the eye discs of "wild type" *D. pseudoobscura* behave in all transplantation experiments like those of the mutant clot (*cl*, Chrom. 2–16.5) of *D. melanogaster*. We concluded that the genetic difference between these two species involves, among others, a mutation at the clot locus. Additional data to support this conclusion are presented in table 1. Among the mutant genes known in *D. melanogaster* there is one, Henna (*Hn* Chrom. 3–23.0), which has a superficial resemblance to both *cl*(m) and +(p). Data are available however, to prove that +(p) is not homologous to *Hn*^{r}(m). Indeed, the eye discs of +(p) implanted into *Hn*^{r}(m) develop a brighter eye color than that developed by +(p) in +(p) and similiar to +(p) implanted into +(m). Moreover, *v*(p) implanted into *Hn*^{r}(m) develops completely non-autonomously, identical with *v*(p) implanted into +(m), while the development of *v*(p) in +(p) is only partly so.

Eosin and white mutants in *D. pseudoobscura* resemble greatly the similarly named mutants in *D. melanogaster*. In both species the white locus is sex-linked and is located in the proximity of the gene Notch, which in its distinct properties is the same in both species (STURTEVANT and TAN 1937). The behavior of *w*^{e}(m) in the transplantation experiments of BEADLE and EPHRUSSI (1936) is similiar to that of +(m). Our experiments within *D. pseudoobscura* show also essentially the same behavior of *w*^{e}(p) and +(p) in respect to autonomous or non-autonomous development. The quantitative differences in our implants of +(p) and *w*^{e}(p) into

v(p), *pr*(p) or p^2(p) are not significant. In the first place if a dilution of eye pigment takes place a slight change in the w^e implants is more difficult to notice than in the "wild type" implants, due to the originally lighter eye color. Consequently, the change to a lighter eye in *v*(p) hosts may be of the same degree in w^e(p) as in +(p) anlagen. In the second place the eye colors of w^e(p), p^2(p) and *pr*(p) implants are much more alike than those of +(p), p^2(p) and *pr*(p). We may conclude that w^e(p) is homologous to w^e(m).

b) The st group [*st*(*p*), *st*(*m*), *cn*(*p*), *cd*(*m*)]. A group of eye color mutants comprising *st*(p), *cn*(p), *or*(p) and *v*(p) shows a great resemblance to the *st*(m), *cd*(m), *cn*(m) and *v*(m) group of *D. melanogaster* mutants. Moreover, the members of each of these groups are so similiar to each other that they can hardly be distinguished by simple inspection. Only *cn*(p) and *cd*(m) show a slight darkening with age, which sets them somewhat apart from the rest. The transplantation methods furnish more critical data for discrimination between these eight mutants. Indeed, the eye discs of *st*(p), *st*(m), *cn*(p) and *cd*(m) implanted into hosts of the + group develop autonomously, whereas *or*(p), *cn*(m), *v*(p) and *v*(m) change in the direction of the "wild type" eye color.

An attempt was made to differentiate between *st*(p), *cn*(p), *st*(m) and *cd*(m) by studying their influence on the production of the v^+ and cn^+ substances. The results are so far not self-consistent. According to BEADLE and EPHRUSSI (1936), *v*(*m*) and *cn*(*m*) develop non-autonomously when implanted into *st*(m) or *cd*(m) hosts. Later, BEADLE and EPHRUSSI (1937) found that *cd*(m) implanted into $w^a v$ (m) fails to change the eye color of the host, but such a change does take place if *st*(m) is implanted into $w^a v$(m) hosts. The implantation of *cd*(m) into $w^a cn$(m) hosts results in the host's eye developing a color intermediate between $w^a cn$ and w^a, but closer to $w^a cn$. Similarly, the implantation of *st*(m) into $w^a cn$(m) hosts changes the eye color of the host to one intermediate between $w^a cn$ and w^a. This intermediate color is apparently closer to w^a than that obtained in the experiments with *cd*(m). These results indicate that *st*(m) supplies more v^+ and cn^+ substances than does *cd*(m). Our experiments within *D. pseudoobscura* showed, at least for the supply of cn^+ substance, the reverse result. Apparently more substance is supplied by *cn*(p) to *or*(p) than by *st*(p). The developing implant eye color of *or*(p) is distinctly lighter in a *st*(p) than in a *cn*(p) host. To check these results we injected body fluid of about one day old pupae of *cn*(p) and *st*(p) into $w^a v$(m) larvae. We found that $w^a v$(m) host flies showed darker w^a-like eyes, if we injected the body fluid of *cn*(p), than in the analogous experiment with *st*(p) body fluid. In the latter case the eyes of the host were like those of $w^a v$(m) flies into which the body fluid from $w^a v$(m) stock itself was injected.

We may conclude that *st*(p), *st*(m), *cn*(p) and *cd*(m) behave alike. Our experiments are not extensive enough to establish a distinct difference between these genes. The presence of the gene clot(m) in *D. pseudoobscura* is of great importance for the amount of v^+ and cn^+ substances. Hence, the darkening effect in *cn*(p) and *cd*(m) indicates the homology of *cn*(p) and *cd*(m). The essentially similar behavior of the remaining comparable genes of this group, namely *st*(p) and *st*(m), indicates that *st*(p) is homologous to *st*(m).

c) The rb group [*rb*(*p*), *g*(*m*), *car*(*m*), *rb*(*m*), *cm*(*m*)]. The pinkish eye colors produced by the *rb*, *ca*, *mg*, and *p* mutants in *D. pseudoobscura* resemble the *rb*, *g*, *car*, *cm*, *ca* and *p* eye colors of *D. melanogaster*. With the aid of transplantation experiments, these mutants can be divided into three distinct classes: the *rb*, *ca* and *pr* groups. The basic difference between these groups is that the implants containing the genes of the *rb* group develop non-autonomously and assume the "wild type" coloration if implanted into hosts carrying genes of the *st* and *v* groups; the implants carrying genes of *ca* and *pr* groups under similar conditions develop autonomously.

Although the possibility is not excluded that *cm*(m) may belong to the *rb* group, the homology between it and *rb*(p) is very doubtful. Indeed, +(p) develops autonomously when implanted into a *cm*(m) host, and in addition the behavior of *cm*(m) and of *rb*(p) is different, both when used as implants and as hosts, in experiments involving *or*(p) and *v*(p).

The behavior of *rb*(m) when implanted into hosts carrying *or*(p) differs from that of the other genes of its group. STURTEVANT and TAN (1937) concluded that "if it be granted that each arm of *pseudoobscura* is equivalent to one of *melanogaster*" (and we have no evidence that this conclusion is wrong) and "if the *pseudoobscura* sequence of the comparable loci" in each arm "is arbitrarily taken as an alphabetical one," then seven inversions are sufficient to turn the sequence in the XL chromosome of *D. pseudoobscura* into that of the X of *D. melanogaster*. They did not include in their calculations the gene *rb*(p). Assuming *rb*(p) to be homologous to *g*(m) or to *car*(m), still only seven inversions are required to get the identical sequence in both species. If we homologize *rb*(p) with *rb*(m) eight steps would be necessary, and more for homology with *cm*(m). Moreover, in the transplantation experiments the behavior of *g*(m) and *car*(m) agrees best with that of *rb*(p). However, a difference between *g*(m) and *car*(m) was obtained in our experiments. Implanted into *mg*(p) hosts, *g*(m) gives rise to an eye color in the implant which is intermediate between a pinkish and a "wild type" color, and indistinguishable from the *rb*(p) eye when implanted into *mg*(p). In the analogous experiment the *car*(m) optic discs develop more nearly autonomously. One additional piece of evidence favors

the homologizing of *rb*(p) with *g*(m) rather than with *car*(m). The gene *car*(m) mutates very rarely, having been recorded only once as a mutant from X-ray treatments, whereas *g*(m) mutates relatively frequently. The mutations at the *rb*(p) locus are not infrequent either. Consequently, we are inclined to believe that *rb*(p) is homologous to *g*(m).

d) The v group [*or*(*p*), *cn*(*m*), *v*(*p*), *v*(*m*)]. The transplantation experiments mentioned above (the *st* group) serve to distinguish the genes *st*(p) *st*(m), *cn*(p) and *cd*(m) from *or*(p), *cn*(m), *v*(p) and *v*(m). These genes have a close resemblance to each other. In our recent paper (GOTTSCHEWSKI and TAN, 1937) we concluded that *or*(p) is homologous to *cn*(m), and *v*(p) to *v*(m). The data presented in table 1 support these conclusions. We may add that in general the *D. pseudoobscura* genes seem to supply less cn^+ and v^+ substances than the *D. melanogaster* genes do. The only exception in *D. melanogaster* is the gene *cl*(m), which behaves like *D. pseudoobscura* "wild type." In *D. pseudoobscura*, the exception seems to be *rb*(p), which supplies at least as much of cn^+ and v^+ substances as the homologous gene in *D. melanogaster*, *g*(m). We may conclude from the data listed under the *rb* and *pr* groups that *or*(p) requires less cn^+, and *v*(p) less v^+ substance than *cn*(m) and *v*(m) respectively. Discussion of these relations is deferred until additional evidence with body fluid is obtained. We homologize *or*(p) with *cn*(m) and *v*(p) with *v*(m).

e) The ca group [*ca*(*p*), *ca*(*m*), *mg*(*p*)]. The characteristics distinguishing the *rb* and *ca* groups have been discussed above. We have presented (1937) the evidence proving that *ca*(p) is homologous to *ca*(m). For the gene *mg*(p) which behaves essentially similarly to *ca*(p), it has been so far impossible to find a homologue in *D. melanogaster; mg*(p) is located in XR(p), *ca*(p) in II(p) and *ca*(m) in IIIR(m), IIIR(m) being homologous to the second chromosome of *D. pseudoobscura*. Since +(m) is non-autonomous in *ca*(p), and behaves similarly in *ca*(m), but is intermediate in *mg*(p), we conclude that *ca*(p) is homologous to *ca*(m).

f) The pr group [*pr*(*p*) *bw*(*m*), p^2(*p*), p^p(*m*)]. The genes *pr*(p) and p^2(p) behave alike with two exceptions. First, it seems that p^2(p) supplies proportionately more cn^+ and less v^+ substances than *pr*(p), because *or*(p) implanted in *pr*(p) shows a change to "wild type" only in small eye pieces. In p^2(p) the effect is always recognizable; *v*(m) becomes more like the "wild type" if implanted into *pr*(p) than if implanted into p^2(p). The other exception is that *pr*(p) in *rb*(p) changes only very slightly, but in analogous experiments with p^2 (p) the effect of *rb*(p) on p^2(p) is more pronounced (see above).

STURTEVANT and TAN (1937) homologized *pr*(p) with *bw*(m), especially because the double recessives *v pr*(p) and *or pr*(p) on one hand, and *v bw*(m) and *cn bw*(m) on the other give almost white eye colors. The optic

discs of *bw*(m) and *pr*(p) give rise in the transplantation experiments to identical eye colors. The four genes of this group behave differently if individuals carrying them are used as hosts for *or*(p), *cn*(m), *v*(p) and *v*(m) implants. The *pr*(p) and *bw*(m) hosts supply proportionately less cn^+ and more v^+ substance than p^2(p) and p^v(m) do. As implants, all four of these genes behave alike. In *pr*(p) and p^2(p) hosts the + anlage develops a color intermediate between + and *pr*(p) and + and p^2(p) respectively, but in *bw*(m) and p^v(m) hosts the development is autonomous. This may be taken as evidence against the assumption that these various genes are homologous. From the facts stated above we are inclined to conclude that *pr*(p) is homologous to *bw*(m) and *p*(p) to *p*(m).

g) The se *group* [se(*p*), se(*m*)]. The colorations of the adult eyes produced by *se*(p) and *se*(m) are similar; no other genes have effects resembling those of the two genes just mentioned. The testicular envelope of *se*(p) and *se*(m) is pale, and the eyes become very much darker with age than they are at hatching. The double recessives *v se*(p) and *v se*(m) have an orange eye color in young flies.

If *se*(p) discs are implanted into *se*(p) hosts, or *se*(m) ones into *se*(m) hosts, the colors of the implants are alike. Likewise, the implantation of *se*(p) and *se*(m) into *v*(p) produces identical eye colors. Nevertheless, if discs carrying genes belonging to the *v* group are implanted into *se*(p), the outcome is different from that observed if *se*(m) hosts are used. A difference in the outcome is observed also when *se*(p) and *se*(m) are implanted into hosts of the + group, or vice versa. Until more information on the nature of the eye pigments in the two species is available, the significance of these differences in the behavior of *se*(p) and *se*(m) is not clear. At present the assumption that *se*(p) is homologous to *se*(m) seems most likely to be true.

DISCUSSION

A list of the assumed homologies between the eye color genes of *D. pseudoobscura* and *D. melanogaster* is presented in table 4. Our results agree throughout with those of DONALD (1936) and STURTEVANT and TAN (1937). So far there is no evidence to show that any genes have been transferred from one chromosome to the other in these species; in other words, translocations seem to be very rare in the evolution process as compared with intra-chromosomal changes, such as inversions. As shown by DOBZHANSKY and TAN (1936) the differences between the gene arrangements in *D. pseudoobscura* and *D. miranda* are also due mainly to inversions, although translocations of short sections have been established in addition.

One difference between these two species in respect to eye color may be that the "wild type" *D. pseudoobscura* "normally" possesses the gene *cl*(m) in homozygous condition. In *D. melanogaster*, on the other hand, the

"wild type" allele of *cl*(m) is "normally" present. In addition, it appears that all *D. pseudoobscura* genes supply less cn^+ and v^+ substances than the homologous genes in *D. melanogaster*. Clot (m) acts like the *pseudoobscura* genes. +(m) and $Hn^r(m)$, for example, although they resemble *cl*(m) closely, differ from it in that they have more v^+ and cn^+ substances. Transplantation experiments with eye anlagen of the double recessives *v cl*(m) and *cn cl*(m) are under way. We expect more conclusive evidence from these experiments. The experiments with body fluid injection will

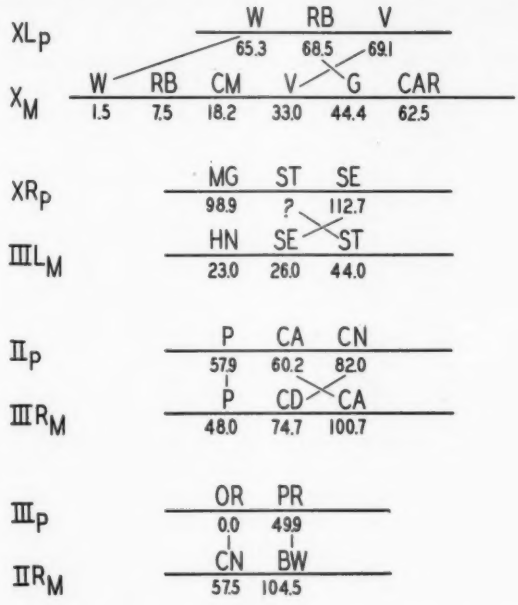

TABLE 4

Homology of the eye color genes in D. pseudoobscura and D. melanogaster (see text).

make clear the relationship of the different substances in the different eye color groups. We may conclude from our experiments to date that the source of cn^+ and v^+ substances in *D. pseudoobscura* is essentially the same as in *D. melanogaster*.

Because in certain cases the implanted anlagen carrying genes of the *rb* group show a change to "wild type" as do *or*(p), *cn*(m), *v*(p) and *v*(m), we conclude that there is a *rb* substance. The evidence that this new substance is not related to the cn^+ and v^+ substances is given (a) by the experiments of +(p) implanted into *rb*(p), *or*(p) or *v*(p); (b) by implants of *or*(p) and *v*(p) into *rb*(p); (c) by the reciprocal experiments of (a) and (b); and (d) by

implants of *pr*(p) and p^2(p) into *rb*(p), *or*(p) or *v*(p). The genes of the + group possess cn^+ and v^+, but no *rb* substance. Orange (p) and *cn*(rn) have v^+ and *rb*, but no cn^+ substance. The genes of the *pr* group possess cn^+ and v^+, but no *rb* substance. Ruby itself also has cn^+ and v^+, but no *rb* substance. It is impossible to construct a step by step change from one of these three substances to another.

BEADLE and EPHRUSSI (1936) assumed originally that the presence of ca^+ substance is the first step toward the formation of v^+ substance, and that the production of v^+ substance is a preliminary step toward cn^+ substance. However, later transplantation experiments of EPHRUSSI and BEADLE (1936), and their experiments with body fluid (1937) have shown

	CN^+	V^+	RB	X
$+_p$	+	+	—	—
ST	+	+	+	—
RB	+	+	—	+
OR	—	+	+	—
V	—	—	+	—
CA	—?	—?	+	+
PR	+	+	—	+

TABLE 5

The presence or absence of cn^+, v^+, rb substances and "X" in the different eye color groups (see text).

that "*ca*" possesses both cn^+ and v^+ substances. The results of our experiments show that a chain of reactions, in the sense that the formation of ca^+ substance is the first step toward the formation of any other substance, is very unlikely. We are inclined to conclude that there is no ca^+ substance at all present, but that in a few groups, of which "*ca*" is an example, something, "X" is concerned, whose presence or absence prevents the optic discs of the + group from developing the "normal" eye color.

It is impossible at present to assume an "*se*" substance. Whenever *se*(p) anlagen are implanted, an entirely new eye color type develops. We defer a discussion of the "*se*" cases until experiments involving the second and the third instars shall have been finished. Two explanations are possible: (a) the analomous behavior of "*se*" optic discs in transplantation

may be the result of differences between host and implant in the period of development at which critical concentrations of the respective substances become available; (b) on the other hand, it is possible, and seems at present more probable, that in the different eye colors, entirely different substances and reactions are involved, giving rise through interactions to a new process.

+(p) is not completely autonomous in *or*(p) and *v*(p), but develops a brighter eye color. This may be due rather to the different relationships of substances and reactions in these types, than to the presence in *or*(p) and *v*(p) of a special substance which changes the "wild type" eye color toward a brighter type.

Table 5 reviews the presence of substances in the different groups. The sign "+" indicates presence, the sign "−" absence. The question marks in the "*ca*" row for cn^+ and v^+ substances indicate that in certain experiments the presence of cn^+ and v^+ substances in "*ca*" is established. The reason that we did not record the *se* group is obvious from our statements above. We did not quote in table 5 the change of *pr*(p) and p^2(p) implanted into *rb*(p), because the non-autonomous development resulted in a new eye color, which was that of neither the host nor the donor. For both latter cases also the statements above are valid.

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SUMMARY

Optic discs can be successfully transplanted from one species of Drosophila to another. The transplanted discs give rise in the interspecific experiments to eyes which always develop autonomously in respect to the size and the color characteristic of its species.

The experiments show that, so far as eye color genes are concerned, no translocations have occurred in the two species since their divergence from a common ancestor.

In both species genes which belong to the same group behave similarly.

We assume that the following genes are homologous: *w*(p)–*w*(m); *cn*(p)–*cd*(m); *st*(p)–*st*(m); *rb*(p)–*g*(m); *v*(p)–*v*(m); *or*(p)–*cn*(m); *ca*(p)–*ca*(m); *pr*(p)–*bw*(m); *p*(p)–*p*(m); *se*(p)–*se*(m). The gene *mg*(p) has no homologous gene in *D. melanogaster*.

"Wild type" *D. pseudoobscura* is in its behavior homologous to *cl*(m). "Wild type" *D. pseudoobscura* and "wild type" *D. melanogaster* differ only quantitatively in the relationships to the various eye color genes.

Our data indicate the presence of at least one new substance, *rb* substance.

The existence of a ca^+ substance seems unlikely. Claret (p), pr(p), p^2(p) and rb(p) prevent the development of "normal" eye color in implanted optic discs of the + group.

The assumption that a chain of substances is present, in such a way that one substance is necessary as a preliminary step to the formation of another substance, seems valid only for cn^+ and v^+ substances.

LITERATURE CITED

BEADLE, G. W., and EPHRUSSI, B., 1936 The differentiation of eye pigments in Drosophila as studied by transplantation. Genetics **21**: 225–247.

1937 Development of eye colors in Drosophila: Diffusible substances and their interrelations. Genetics **22**: 76–86.

DOBZHANSKY, TH., and BAUER, H., 1937 A comparison of gene arrangement in *Drosophila azteca* and *D. athabasca*. Genetics **22**: 185.

DOBZHANSKY, TH., and TAN, C. C., 1936 Studies on hybrid sterility III. A comparison of the gene arrangement in two species, *Drosophila pseudoobscura* and *Drosophila miranda*. Z. i. A. V. **72**: 88–114.

DONALD, H. P., 1936 On the genetical constitution of *Drosophila pseudoobscura*, race A. J. Genet. **33**: 103–122.

EPHRUSSI, B., and BEADLE, G. W., 1936 Development of eye colors in Drosophila: Studies on the mutant claret. J. Genet. **33**: 407–410.

1937 Développement des couleurs des yeux chez la Drosophile: Revue des expériences de transplantation. Bull. Biol. **71**: 54–74.

GOTTSCHEWSKI, G., and TAN, C. C., 1937 Die Homologie der Augenfarbgene von *Drosophila melanogaster* und *Drosophila pseudoobscura* bestimmt durch das Transplantationsexperiment. Biol. Zbl. **57**: 273–282.

MORGAN, T. H., BRIDGES, C. B., and STURTEVANT, A. H., 1925 The genetics of Drosophila. Bibliographica Genetica **2**: 262 pp.

STURTEVANT, A. H., and TAN, C. C., 1937 The comparative genetics of *Drosophila pseudoobscura* and *Drosophila melanogaster*. J. Genet. **34**: 415–432.

GENETICS OF NATURAL POPULATIONS. I. CHROMOSOME VARIATION IN POPULATIONS OF DROSOPHILA PSEUDOOBSCURA INHABITING ISOLATED MOUNTAIN RANGES

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INTRODUCTION

IN RECENT years there appears to be a growing interest in the genetics of free-living populations, a subject hitherto almost untouched. Certain species of Drosophila prove to be very suitable for investigations in this field. TSCHETWERIKOFF (CHETVERIKOV), DUBININ and their collaborators in Russia, TIMOFEEFF-RESSOVSKY in Germany, GORDON in England, and STURTEVANT in this country have secured much valuable information which opens new vistas and raises a host of new problems (for a short review of the literature see DOBZHANSKY 1937). It has seemed to us that a comparison of the genetic constitutions of several free-living populations from the same general region, and yet isolated from each other, may be of interest. Samples of the populations of *Drosophila pseudoobscura* inhabiting island-like mountain forests in the Death Valley region of California and Nevada were collected. This region is especially favorable for our purposes, since it is as yet practically undisturbed by man's activities.

We wish to acknowledge our obligations to Professor A. H. STURTEVANT for his valuable advice, to Mr. H. D. CURRY whose help enabled us to collect material in certain rather inaccessible localities, and to Messrs. G. T. RUDKIN and EDWARD HELD for their assistance in conducting the experiments.

MATERIAL

The nature and source of the material are so important in the present investigation that we are forced to consider them more carefully than is customary in genetic accounts.

East of the rampart of the Sierra Nevada, in California and the adjacent part of the state of Nevada, there lies an extremely arid desert plateau. This plateau is strewn with mountain ranges and furrowed by deep valleys, extending mostly in the meridional direction (fig. 1). Some of the ranges lift their crests much above six thousand feet, and their upper reaches are covered by open forests consisting of piñon (*Pinus monophylla*), Juniperus, oak, and (on Charleston Peak and the Sierra Nevada), western yellow pine (*Pinus ponderosa*) and other conifers. The valleys intervening

between the ranges are barren except for a very xerophytic vegetation. The mountain forests may, consequently, be described as a series of islands surrounded by a sea of deserts. The contrast between the climatic and ecological conditions encountered on the mountain tops and in the valleys is exceedingly sharp.

The biology of *Drosophila pseudoobscura* in the natural state is very little known. According to the observations of DUDA (1924) and of STURTEVANT (unpublished), its relatives (*D. obscura*, *D. affinis* and other species) feed on the fermenting sap of bleeding trees, and the same is probably true for *D. pseudoobscura*. At any rate, the latter species is restricted to regions having some tree vegetation; occasionally it may be trapped a few thousand feet from the nearest trees, but it has never been found in terrain having only grass vegetation. In arid regions *D. pseudoobscura* is confined to mountains where forests can grow, and in general the denser the forest the more abundant is the fly population. To what extent populations inhabiting different mountain ranges exchange individuals it is very difficult to tell. During the warm season of the year the desert appears to be a formidable obstacle to the distribution of the fly, but an occasional transfer of a stray individual by wind or other agents is not excluded. At the very best, a migration of flies within a continuously forested region is easier than from one range to another across desert valleys. The population inhabiting each mountain range is largely, if not completely, isolated from others.

Samples of the population of *D. pseudoobscura* have been collected in eleven localities shown on the map (fig. 1). Each locality corresponds to a forested mountain range, and in the area studied there exist only two or three ranges where collecting has not been attempted. The entire collecting was accomplished within two months, from the middle of May to the middle of July, 1937; the genetic analysis of the samples has been carried on as nearly simultaneously as possible. The following technique has been adopted. Ten to twenty trap bottles with fermenting banana mush were exposed in each locality, the distance between the traps farthest apart being no less than a quarter and no more than one mile. The traps were left out for a few hours around sunset, and in some cases overnight. The flies that entered the traps were placed in vials with food and transported without delay to the laboratory. In the following presentation each sample of flies is referred to by the name of the locality (island forest) in which it was collected. It must be emphasized, however, that each sample comes from only a small fraction of the area of a given forest; the possibility that the populations inhabiting different parts of the same forest may be different is by no means excluded.

THE GENE ARRANGEMENT IN THE THIRD CHROMOSOME

Strains of *D. pseudoobscura* coming from the same or from different localities are frequently unlike in the gene arrangement. The third chromosome proves to be more variable in this respect than the rest; seventeen arrangements related to each other as single or multiple inversions have

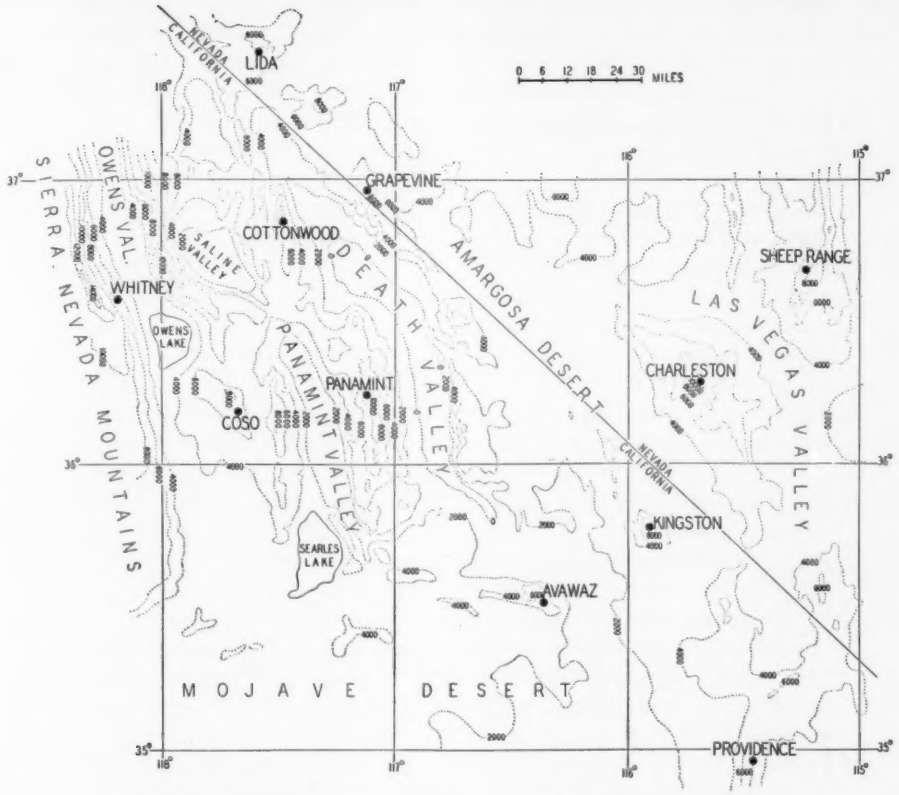

FIGURE 1. Map showing localities from which collections were made. (Note: Avawaz should be spelled Awavaz as in text.)

been recorded (STURTEVANT and DOBZHANSKY 1936a; DOBZHANSKY and STURTEVANT 1938). Populations inhabiting the mountain ranges in the Death Valley region also proved to be variable in the gene arrangement, and the variability observed is of a kind that permits certain inferences as to the relation of these populations to each other.

The gene arrangement in a given individual can be determined by

inspection of the chromosomes in the cells of the salivary glands. In structurally homozygous individuals the distribution of the stainable discs in the chromosomes reflects the arrangement involved; if two homologous chromosomes differing in the gene arrangement are present, a characteristic pairing configuration results. For descriptions of each of the seventeen arrangements the paper by DOBZHANSKY and STURTEVANT (1938) may be consulted.

Since females caught outdoors are usually fertilized already, they were placed in individual cultures and allowed to produce offspring. Wild males were crossed to females homozygous for the third chromosome recessives orange (*or*) and purple (*pr*), and having the standard gene arrangement in the third and in all other chromosomes. From the offspring of each wild female a single larva was taken, and a temporary acetocarmine preparation of its salivary glands was made. Such a larva carries one maternal and one paternal third chromosome, and therefore an examination of its nuclei furnishes the information on the gene arrangement in two chromosomes present in the wild population in question. From each male culture seven larvae were taken, and one salivary gland from each larva was fixed and stained in acetocarmine. If the male parent contained two third chromosomes similar in the gene arrangement, all seven larvae show identical chromosome configurations; if two different chromosomes were present, the offspring fall into two equally numerous classes, and the probability is sufficiently great that among seven larvae both classes are represented (only one out of sixty-four heterozygotes is thus not detected). The third chromosomes were carefully examined in every preparation, the rest receiving only a cursory inspection.

Four previously known gene arrangements have been found in populations coming from the localities shown in figure 1. These have been designated as "Standard," "Arrowhead," "Chiricahua," and "Mammoth" respectively (DOBZHANSKY and STURTEVANT 1928). In the offspring of a female collected on Kingston Peak there was found a single larva showing a fifth, and previously unknown, arrangement in the third chromosome. The culture was lost before the new arrangement could be studied in detail. Table 1 shows the frequencies of the four arrangements in the eleven populations; each observed frequency is accompanied by its probable error. The total numbers of the chromosomes examined are given in the rightmost column.

An examination of table 1 shows that the Arrowhead, Chiricahua, and Standard arrangements are present in every population, Arrowhead being much the commonest. The Mammoth arrangement was found in only two localities, a single chromosome in each case. Its frequency is therefore negligible. Nevertheless, some of the populations are significantly different

from others in the relative frequency of the different arrangements. Only three localities, namely Panamint, Awavaz, and Charleston, are inhabited by populations that seem to be identical in this respect. The Coso population is slightly but significantly different from the preceding three. The remaining six populations differ significantly from each other as well as from the four mentioned above, although the sample from Mt. Whitney is too small to be much relied upon.

TABLE 1

Frequencies (in percent) of the four gene arrangements encountered in the third chromosome.

MOUNTAIN RANGE	ARROWHEAD	CHIRICAHUA	STANDARD	MAMMOTH	CHROMOSOMES TESTED
Lida (Mt. Magruder)	76.80±1.80	6.00±1.01	16.80±1.59	0.40	250
Mt. Whitney	69.57±4.57	8.70±2.73	21.73±4.10	—	46
Coso	72.27±1.89	14.45±1.48	13.28±1.43	—	256
Cottonwood	51.20±2.13	9.60±1.25	38.80±2.08	0.40	250
Grapevine (Funeral)	50.86±2.23	18.70±1.73	30.43±2.04	—	230
Panamint (Telescope)	67.41±2.11	18.75±1.75	13.83±1.56	—	224
Awavaz	62.20±3.61	19.51±2.95	18.29±2.88	—	82
Kingston	64.08±2.25	5.34±1.06	30.58±2.17	—	206
Charleston	68.75±1.96	19.14±1.66	12.11±1.38	—	256
Sheep Range	88.11±1.53	1.98±0.64	9.90±1.42	—	202
Providence	82.00±1.50	10.00±1.17	8.00±1.06	—	300

A more detailed analysis of the data presented in table 1 brings to light some additional facts. It must be borne in mind that each of the three arrangements commonly found in the geographical region now under study is encountered also beyond its confines (DOBZHANSKY and STURTEVANT 1928). The Arrowhead arrangement is present throughout the distribution area of the species, except in southern Mexico. The populations inhabiting New Mexico, southern Colorado, northern Arizona, and most of southern Utah show a hundred percent frequency of Arrowhead; westward and north-westward from there the frequency of Arrowhead declines, and reaches a minimum on the Pacific Coast. Standard shows the opposite regularity: its frequency is highest on the Pacific Coast, and decreases as one proceeds eastward, reaching zero just east of the Death Valley region. Finally, Chiricahua is a southern type, very common in Mexico, and the northernmost locality where it has been recorded is in the Toyabe Range, Nevada, lying less than a hundred miles north of Lida. Are, then, the differences observed between the relatively closely adjacent localities in the region studied by us merely an expression of the general geographic trends in the distributions of the gene arrangements? If that were so, one might expect the frequency of Standard to be greatest in the western or

north-western localities, and to decrease as one proceeds eastward. A similar gradient in the direction south-north would be expected for Chiricahua, and an east-west gradient for Arrowhead. Indeed, gradients of this kind have been observed by many students of geographical variation in all sorts of animals and plants (for example, SUMNER 1929).

An examination of table 1 in conjunction with figure 1 reveals however no definite gradients. The greatest frequency of Standard obtains in the centrally located Cottonwood mountains, and decreases westward as well as eastward. Chiricahua is more frequent in the center (Awavaz, Panamint, Charleston) than it is in the south (Providence). Arrowhead is most frequent on the fringes of our region, and least frequent in the center. Not only is there no regularity if our region is treated as a part of the general species area, but there are no definite trends within the region itself. Mountain ranges that lie far apart may be more similar than adjacent ones. Thus, Charleston and Awavaz are alike, while Kingston which is located almost exactly half way between the two is distinctly different from either. The frequency of the Chiricahua arrangement is very low on Kingston Peak and Sheep Range, and high on Charleston which lies between them. Panamint is similar to the rather remote Awavaz and Charleston mountains, and very different from the Cottonwood mountains which represent merely a northern extension of the Panamint. Sheep Range and Providence are more similar to each other and to the remote Lida than to the intervening Charleston and Kingston Peaks. In short, the variation seems to be random. The possible significance of this fact is discussed below.

The data on the gene arrangement in the third chromosome were collected in such a way that in every case two chromosomes present in a given individual were examined. This permits us to study the relative frequencies in natural populations of individuals homozygous and heterozygous for the different gene arrangements. Let the gametic frequencies of the Arrowhead, Chiricahua, and Standard arrangements be a, c, and s respectively ($a+c+s=1$). If mating is random, and the viabilities of the structural homo- and heterozygotes are alike, the expected frequencies of the different types are as follows:

Homogyzous Arrowhead—a^2
Homogyzous Chiricahua—c^2
Homogyzous Standard—s^2
Heterozygous Arrowhead/Chiricahua—$2ac$
Heterozygous Arrowhead/Standard—$2\ as$
Heterozygous Chiricahua/Standard—$2\ cs$

A comparison of the observed and expected frequencies is presented in table 2. In general, the two sets of values agree very well, attesting that

TABLE 2

The observed and expected frequencies of the inversion homozygotes and heterozygotes in various populations.

LOCALITY		HOMOZYGOTES			HETEROZYGOTES		
		ARROWHEAD	CHIRICAHUA	STANDARD	ARROWHEAD CHIRICAHUA	ARROWHEAD STANDARD	CHIRICAHUA STANDARD
Lida	Obs.	74	—	6	14	29	1
	Exp.	73.75	0.45	3.5	11.5	32.2	2.5
Coso	Obs.	70	2	6	28	17	5
	Exp.	66.85	2.7	2.25	26.7	24.6	5.0
Cottonwood	Obs.	34	2	21	13	47	7
	Exp.	32.75	1.5	18.9	12.3	49.6	9.3
Grapevine	Obs.	27	3	12	27	36	10
	Exp.	29.75	4.0	10.65	21.9	35.5	13.1
Panamint	Obs.	49	3	3	32	21	4
	Exp.	50.8	3.9	2.1	28.2	20.9	5.8
Kingston	Obs.	45	1	12	6	36	3
	Exp.	42.3	0.3	9.6	7.1	40.4	3.4
Charleston	Obs.	62	7	5	33	19	2
	Exp.	60.5	4.7	1.9	33.7	21.3	5.9
Sheep Range	Obs.	77	—	—	4	20	—
	Exp.	78.4	0.04	0.99	3.5	17.6	0.04
Providence	Obs.	102	3	2	22	20	1
	Exp.	100.9	1.5	1.0	24.6	19.7	2.4

the above assumptions of the randomness of mating and equal viability are correct. A more detailed examination of table 2 shows however that the observed figures for homozygotes are somewhat more frequently higher than lower than the expected ones, and the reverse is true for the heterozygotes. The differences are in no case statistically significant. This small discrepancy, if real, may have two explanations. First of all, a small fraction of the heterozygotes might have been misclassified as homozygotes (see above). Secondly, the fly samples from each locality were collected in a number of trap bottles placed at some distance from each other. If the flies spend their life in the immediate vicinity of the place where they were born, the mating may not be absolutely random, and a certain amount of inbreeding may be involved. This latter possibility is theoretically very interesting, but no conclusions can be drawn from the data now available. What is demonstrated is merely that the natural populations are not segregated into non-interbreeding sections each of which has a given gene arrangement.

THE "SEX-RATIO"

Certain males of *D. pseudoobscura* produce, when crossed to females of any genetic constitution, mostly or only daughters and few or no sons.

This peculiar "sex-ratio" condition is known to be inherited through the X chromosome, and to be associated with an inversion in the right limb of the latter, the association apparently being invariable. The "sex-ratio" is often encountered in wild populations of *D. pseudoobscura*, but the frequency is higher in the southern than in the northern part of the specific area (STURTEVANT and DOBZHANSKY 1936b). It proved to be rather common in most of the populations from the Death Valley region.

To detect the presence or absence of the "sex-ratio," wild males or single sons of wild females were crossed in individual cultures to two or three *or pr* females. In the F_1 generation some of the cultures produced few or no sons, indicating that the father carried the "sex-ratio" in its X chromosome. Complete counts are unnecessary to distinguish the "sex-ratio" from the normal cultures, because in the former only very few or no males are found. Since the salivary gland chromosomes of the F_1 larvae were studied in order to determine the gene arrangement in the third and the other chromosomes, some "sex-ratio" cultures were in fact detected before any adult flies appeared in them. Nevertheless, no special attention was paid to the presence or absence of the "sex-ratio" inversion in the right limb of the X chromosome in these cultures, so that the statistical data of the frequency of the "sex-ratio" presented in table 3 are based on the genetical and not on cytological determinations.

TABLE 3

Frequency (in percent) of the "sex-ratio" condition in the X chromosome.

LOCALITY	ELEVATION (FEET)	DATE OF COLLECTING	SEX-RATIO	CHROMOSOMES TESTED
Awavaz	5000	May 22	23.08	26
Providence	5000?	May 7, 8	16.06	137
Sheep Range	6000	June 5	11.57	121
Kingston	4800	May 21	11.38	123
Cottonwood	7800	June 23, 24	10.66	122
Lida	8000	June 15, 17	9.09	66
Grapevine	7500	June 22	8.33	72
Panamint	8300	May 20	8.09	136
Charleston	8200	June 4	6.72	119
Coso	6500	July 14, 15	4.70	149

From 4.7 percent (in Coso) to 23.1 percent (in Awavaz mountains) of the X chromosomes were found to carry the "sex-ratio." Table 3 shows also the approximate elevation above sea level of the localities in which the collecting was done, and the date of collecting. There seems to be a weak negative correlation between the elevation and the frequency of the "sex-ratio"; the existence of such a correlation has been suspected on the basis of another set of data (STURTEVANT and DOBZHANSKY 1936b).

Taken at their face value, the figures in table 3 suggest that the frequency of the "sex-ratio" in localities of equal elevation diminishes as the season advances, but the reality of this relationship is certainly far from established.

CHROMOSOMAL VARIATION IN RACE B

All of the above data concern race A of *D. pseudoobscura*. Race B of the same species has been found only in two out of the eleven localities shown in figure 1, namely on Mt. Whitney and in Coso mountains. On Mt. Whitney more than three times as many race B as race A flies came into the traps, while in Coso 3 race B individuals were encountered together with 149 of race A. The geographical distribution of race B lies west and north-west from the Death Valley region, Coso mountains being in fact the extreme south-eastern locality where this race has been thus far encountered.

The gene arrangement was studied in 74 race B third chromosomes from Mt. Whitney and in 6 chromosomes from Coso. On Mt. Whitney 15 chromosomes, or 20.3 percent, showed the Standard, and 1, or 1.35 percent, the Klamath arrangement. The remaining 58, or 78.4 percent, of the chromosomes had a gene arrangement which has not been encountered previously. This new arrangement, designated as "Whitney," differs in a single inversion from Klamath and in two inversions from the Standard (Klamath and Standard differ in a single inversion). The distal end of the Whitney inversion lies proximally from the distal end of the Klamath one, and the proximal end of Whitney lies nearer to the base of the chromosome (section 69A) than in Klamath. The gene arrangement in Whitney is therefore on the whole more similar to that in the Standard than to that in Klamath. Out of the six chromosomes from Coso, three had the Whitney and three the Standard arrangement.

The discovery of a new arrangement in the third chromosome of *D. pseudoobscura* (the eighteenth in the species) is in itself not surprising. What is more unexpected is that the new arrangement has been found to be so frequent on Mt. Whitney, on the eastern slope of the Sierra Nevada, while it has not been encountered at all among race B individuals from the Sequoia National Park (Giant Forest and Lodgepole Camp), from which samples of flies had been previously studied (DOBZHANSKY and STURTEVANT 1938). The Giant Forest and Lodgepole Camp localities lie less than a hundred miles from Mt. Whitney, on the eastern slope of the Sierra Nevada. On the contrary, two other gene arrangements known from Sequoia Park have not been found on Mt. Whitney. This suggests that populations of *D. pseudoobscura* are very local, and that few migrants pass from one locality to the other. One must note however that the Giant Forest and Mt. Whitney localities are separated by the alpine desert of

the highest part of the Sierra Nevada, which may constitute a natural barrier for the distribution of *D. pseudoobscura*.

DISCUSSION

It has been shown above that three distinct gene arrangements in the third chromosome, namely Standard, Arrowhead, and Chiricahua, are encountered in every one of the eleven populations studied. The relative frequencies of these arrangements, however, vary within rather wide limits; there is no geographical trend or regularity in these variations, so that populations from adjacent localities may be more different from each other than populations from remote ones. In order to evaluate properly the significance of these observations one must recall certain other facts discussed in detail by DOBZHANSKY and STURTEVANT (1938). The gene arrangements known in the third chromosome of *D. pseudoobscura* are members of a single phylogenetic series. Some arrangements differ from others in a single inversion, while others are related only through a chain of discrete intermediate forms, each link in the chain representing a single inversion step. Thus, Arrowhead and Standard differ in a single inversion, while it takes three inversions to pass from Standard to Chiricahua, four to derive Chiricahua from Arrowhead, three to derive Mammoth from Standard, and two to get Mammoth from Chiricahua, or vice versa. It is significant that in the mountains of the Death Valley region we find only the gene arrangements just named, and do not find the intermediates between them (which, however, are encountered in other, more or less remote, geographical localities). This means that the variety of the gene arrangements now observed in the Death Valley region has arisen elsewhere, or at any rate that its origin has taken place at a certain more or less remote time. Only Standard and Arrowhead may be conceived to transform into each other by "mutation," although we not only lack evidence that they do so regularly, but even have reasons to doubt that this is the case.

What are, then, the driving causes of the historical process which has resulted in the differentiation of the populations inhabiting separate mountain ranges? Since the differences observed are merely quantitative, and since, taken as a whole, the Death Valley populations are intermediate between populations living in the surrounding territories, the possibility of migration as one of the causes must be considered. Arrowhead is more frequent to the east and less frequent to the west of the Death Valley region than anywhere in that region itself. For Standard the relations are the reverse of those for Arrowhead, while Chiricahua is most frequent to the south of Death Valley. If the rate of exchange of individuals between populations inhabiting parts of the distribution area of the species is great

enough, a chain of populations intermediate between the extremes will result. By "great enough" we mean a migration rate sufficient to outweigh other causes leading toward diversification of local populations. The populations inhabiting the Death Valley region form, however, no geographical chains linking together the populations of the surrounding country. We have seen that the characteristics of a population inhabiting a given mountain range are independent of those of the populations in the neighboring mountains. Each population seems to be sufficiently isolated from the others, and migration, if it occurs at all, is not a factor of paramount importance in determining the characteristics of a colony.

The possibility that the gene arrangement may not be indifferent for the viability of its carrier, and that selection has caused one or the other arrangement to be frequent on a given mountain range, must be considered. The localities where collecting was done differ in altitude, climate, flora, and other conditions. The frequency of "sex-ratio" shows indeed a suggestive correlation with the altitude of the locality. The "sex-ratio" cannot, however, be likened to the variations in the gene arrangement in the third chromosome; while the former produces an easily discernible effect, namely unisexual progenies, the latter do not seem to be correlated with any physiological or morphological characteristics of their carriers. There is also no trace of a correlation between the frequency of any gene arrangement in a given locality and any peculiarities of the latter. Since, furthermore, every one of the three gene arrangements is present in every locality, and presumably has been indigenous there for countless generations, the supposition that these chromosome structures are subject to selection is extremely improbable, although, by the very nature of things, it cannot be completely excluded.

By far the most probable explanation of the observed differences between populations of the separate mountain ranges is that the frequency of a gene or a chromosome structure is subject to random fluctuations. WRIGHT (1931, and other works) has shown that, theoretically, such fluctuations must occur in populations whose size is not infinitely large, and that the smaller the population the greater is the amplitude of the fluctuations in a given time interval. In a species segregated in numerous colonies this process will sooner or later lead to a differentiation, provided only that its intercolonial migration rate is not great enough to cancel the effects of the fluctuations. The mountain ranges of the Death Valley region, isolated from each other by the intervening deserts, furnish a set of conditions that might favor such a differentiation. Suppose, for example, that this region was inhabited originally by a homogeneous population in which the frequencies of the three commonest gene arrangements were like those now found in Panamint, Awavaz, and Charleston mountains

(fig. 1 and table 1). A segregation of this population into colonies restricted each to an isolated mountain forest might have resulted in the course of time in a diversification. In some populations, like those inhabiting the three mountain ranges just named, the original frequencies have been retained. Other populations have deviated to a smaller or greater extent from the original condition. But there is not, nor is there expected to be, any geographical trend or regularity in those fluctuations. Adjacent mountains are as likely to retain as they are to lose the similarity in the composition of their populations.

The factor that is decisive for an evaluation of the above hypothesis is how large is the effective size of the breeding population in the colonies of the flies inhabiting each mountain range? The greater the size, the shorter the time interval during which this differentiation may be supposed to have taken place, the less probable is the hypothesis, and vice versa. We hope to be able to present some information bearing on this problem at a later date.

SUMMARY

1. *Drosophila pseudoobscura* inhabits mountain forests in the Death Valley region, but not the intervening deserts. Samples of the fly populations were collected in eleven localities (fig. 1).

2. Three different gene arrangements in the third chromosome are present in every population studied. The difference between these arrangements is due to inversion of chromosome sections.

3. Inversion heterozygotes and homozygotes are present in every population in theoretically predictable proportions (table 2).

4. The relative frequency of each arrangement varies from locality to locality (table 1). Only three out of the eleven localities are inhabited by populations that seem to be identical with respect to the relative frequencies of the gene arrangements.

5. Populations inhabiting adjacent localities are no more likely to be similar than populations from remote ones.

6. It is concluded that the rate of migration of flies between localities is small. The genetic compositions of the populations inhabiting different localities may drift apart in the course of time.

7. A certain proportion of the X chromosomes carry the "sex-ratio" condition (table 3). There is a slight correlation between the frequency of the "sex-ratio" and the elevation of the locality above sea level.

LITERATURE CITED

DOBZHANSKY, TH., 1937 Genetics and the origin of species. IX+364 pp. New York: Columbia University Press.

DOBZHANSKY, TH., and STURTEVANT, A. H., 1938 Variations in the gene arrangement in the chromosomes of *Drosophila pseudoobscura*. Genetics **23**: 28–64.

DUDA, O., 1924 Revision der europaischen Arten der Gattung Drosophila Fallen (Dipt.). Entomol. Meddelelser **14**: 246–313.

STURTEVANT, A. H., and DOBZHANSKY, TH., 1936a Inversions in the third chromosome of wild races of *Drosophila pseudoobscura*, and their use in the study of the history of the species. Proc. Nat. Acad. Sci. **22**: 448–450.

STURTEVANT, A. H., and DOBZHANSKY, TH., 1936b Geographical distribution and cytology of "sex ratio" in *Drosophila pseudoobscura* and related species. Genetics **21**: 473–490.

SUMNER, F. B., 1929 The analysis of a concrete case of intergradation between two subspecies. Proc. Nat. Acad. Sci. **15**: 110–120, 481–493.

WRIGHT, S., 1931 Evolution in Mendelian populations. Genetics **16**: 97–159.